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THE PATHOGENESIS OF EXPERIMENTAL SILICOTIC FIBROSIS

by

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with 16 illustrations

Submitted: April, 1954.

Abstract

The present investigation was undertaken to test some of the hypotheses concerning the etiology of pneumoconiosis. Different modifications of particulate silica such as tridymite, quartz and amorphous silica were used for inducing silicotic fibrosis in the lung, in the testis and in the muscular tissue of the rat. The genesis of the silicotic nodule was studied histologically. The action of protective substances such as aluminium and carbon was also studied. A new hypothesis about the etiological factors responsible for the formation of the silicotic nodules and for the action of protective substances, based on the adsorption phenomenon, is advanced.

CONTENTS

THE PATHOGENESIS OF EXPERIMENTAL SILICOTIC FIBROSIS

INTRODUCTION

In the development of the theories about the pathogenesis of silicotic fibrosis, it is interesting to note that controversy about the basic cause has from the start centered around physical or chemical theories. Mavrogordato (1918) believed that the connective tissue proliferation was due to the *physical character* of the silica crystal. He assumed that when the particles were inhaled, their sharp edges continuously irritated the tissue in special loci and consequently gave rise to progressive fibrous tissue proliferation. This theory, however, was abandoned in favour of the *chemical theory* after the experimental evidence of Kettle (1932) came to hand. The chemical or solubility theory maintained that silica in solution is a toxic substance which causes cell necrosis and a chronic inflammatory condition, factors which predispose to fibrosis. The results of King (1947), however, demonstrated that the parallelism between solubility and toxicity of silica derivatives is not constant. In fact, as far as solubility and toxicity are concerned, the least soluble substances are generally the most harmful. This theory is therefore not valid.

The *supersonic wave theory* as proposed by Permeggiani (1947) is strongly supported by the experimental evidence of Evans (1948). The results of Evans, however, were obtained under such abnormal physiological conditions that this theory can not be accepted without reserve. The ion exchange mechanism of Policard (1946) has also certain limitations as an explanation of the cause of silicotic fibrosis.

Velicogna (1946) suggested that silicotic fibrosis is due to piezoelectric or pyroelectric phenomena. These characteristics are only exhibited by minerals with an asymmetric crystalline structure. According to Permeggiani (1947), the pyroelectric phenomenon is not concerned in the pathogenesis of fibrous tissue because the particulate matter in the lung is kept at a constant temperature. To what extent piezoelectricity is involved as an etiological factor in silicotic fibrosis can only be determined by further experimental work.

At present the evidence on the morphology, as well as that on the specific locus of the fibrotic nodule in the lung tissue, is contradictory. The confusing results can only be explained by the fact that very few cases of early silicosis were available for postmortem examinations. The early silicotic nodules all develop simultaneously and the condition is usually complicated in the later stages by additional tuberculous infiltration. (King and Belt, 1938).

Apart from these problems the role of *protective substances* in the prevention of silicosis is also not very well understood. Mavrogordato (1918) made the important observation that when carbon and flint particles were simultaneously administered the carbon stimulated the phagocytosis and subsequent elimination of the flint, provided that there was considerably less flint than carbon. Further proof was provided by the experimental work of Hayes (1926) and Denny, Robson and Irwin (1939), that an efficient

antidote to silicosis might be established. Today *aluminium therapy* is quite commonly used (Robson, 1944; King, et al., 1950; Hannon, 1944, 1946), although it is still uncertain how this protection is achieved.

The present investigation was therefore undertaken with a view to elucidating these different aspects of the pneumoconiosis problem. The following were the main subjects of study in this connection:

(1) The capacity of piezoelectric and non-piezoelectric minerals to induce silicosis and also the validity or otherwise of the piezoelectric hypothesis in the pathogenesis of silicotic fibrosis (Velicogna, 1946).

(2) A histological study of the genesis of the silicotic nodule and its development in the lung tissue correlated with the blood picture.

(3) The genesis and development of fibrotic nodules in testicular and muscular tissue.

(4) The influence of protective substances on the pathogenesis of silicotic nodules in the lung.

(5) The adsorptive capacity of known pneumoconiosis-producing substances as well as of such substances as were suspected of having pneumoconiotic properties. In connection with the evidence obtained in this experiment *a new hypothesis about the pathogenesis of silicotic fibrosis is put forward.*

MATERIAL AND METHODS

All experiments were carried out on albino rats, Rattus Norwegicus, Wistar Institute, bred in the Department of Physiology, University of Stellenbosch.

The mineral material was obtained from Prof. D. L. Scholtz of the Geology Department of the University of Stellenbosch and the material was ground in a ball mill of the author's own design until the average particle size was approximately 6 μ. The material was purified by boiling with concentrated hydrochloric acid for two hours and afterwards washing with distilled water till neutral towards litmus. Tridymite was prepared from amorphous silica by heating the material to 1,400° C for four hours (Partington, 1949).

The particles were suspended in distilled water and sterilized. The suspension was administered according to the technique of Kettle and Hilton (1932) by direct intratracheal injection. The anaesthetic used was ether, and the operation was performed under aseptic conditions. The administration of the suspensions in testicular and muscular tissue was attained by slow injection to avoid tissue destruction.

Experimental animals were all killed by ether anaesthesia, the thorax opened by two dorso-lateral incisions and the trachea and lung tissue carefully dissected. The trachea was usually ligatured to prevent blood from entering the lungs. The lungs were injected along the trachea with 2ml. of fixation fluid (4—5% formalin in 0.9% NaCl), and immediately after dissection fixed in the same solution. Parts of other organs were also fixed in formalin and stained with haematoxylin and eosin after serial sections had

been made. For connective tissue the preparations were stained by the azan technique. Imprints of lung tissue were made according to Gillman and Gillman (1949) and stained with Leishmann stain. In cases where differential white cell counts were made, an average of 500 cells were enumerated on each slide. The adsorptive capacity of the different minerals was determined by their ability to adsorb chorion gonadotrophin from pregnancy urine.

RESULTS

The results obtained in a preliminary investigation with the direct intratracheal injection method, and the dust chamber method, indicated that the former method was better than the latter. This method was therefore exclusively used in the later investigations.

EXPERIMENT I. **A comparative study of piezoelectric and non-piezoelectric minerals for the induction of silicosis.**

In this experiment 48 rats were used (24 females and 24 males). They were divided into three groups A, B and C, with 8 females and 8 males in each group. Each rat of group A received an intratracheal injection of 1 ml. quartz suspension (50 mg. per ml. sol.). *Qwartz is a piezoelectric substance and the influence of piezoelectricity on fibrogenesis could therefore be determined in this group.*

The animals in group B were intratracheally injected with 1 ml. tridymite suspension (50 mg. per ml. sol.). This silica derivative has no piezoelectric activity; *in other respects it has the same characteristics as quartz.* The animals of group C each received an injection of 1 ml. *amorphous silica suspension* (50 mg. per ml.). This substance is *an amorphous powder without any piezoelectric properties.*

Animals of each group were killed at set intervals over a period of 360 days.

In the postmortem investigation a striking difference was observed between the external appearance of the lungs of the experimental animals as compared with the normal rat lung. The experimental lungs were much bigger, they did not collapse at all and conspicuous tubercles with scattered petechiae in between were observed on their surfaces. The histological picture of the lungs of groups A, B and C, revealed only a slight difference in the degree of fibrous reaction to the different silicates. At an early stage an extraordinary *cell infiltration was noted in the alveoli* consisting mainly of polymorphonuclear cells, mononuclear cells and phagocytes. The cell infiltration was accompanied by *a thickening of the septal walls and proliferation of the bronchial epithelium.* A drastic interference in the blood supply was noticed and congestion was widespread. Blood was found in the alveolar tissue and bronchioli. Congestion, blood stasis, cell infiltration and edema were evidently primary reactions of the lung tissue towards quartz, tridymite and amorphous silica.

In all three groups a hyperplastic proliferation of the bronchial epithelium was noticed (Figure 1). This hypertrophy was accompanied by mucin secretion which evidently caused bronchiolar obstruction and respiratory dysfunction. In some cases the bronchiolar passages were totally obliterated.

The hypertrophied bronchial epithelium began to degenerate after a while and especially in the experimental animals of group C considerable destruction of this tissue was observed. This destruction was succeeded by connective tissue proliferation in the bronchial tree especially at those sites where it had been in closest contact with the particulate matter. This tissue response was more striking with amorphous silica than with either quartz or tridymite (Fig. 2). It was evident that the connective tissue response to amorphous silica was mainly limited to the bronchial tree while quartz and tridymite also affected the alveolar tissue.

In the later stages of the experiment the difference in lung response to quartz and tridymite on the one hand and amorphous silica on the other hand was still more striking than in the earlier stages. In both quartz and tridymite groups there was progressive fibrous proliferation with eventual development of concentric fibrous nodules not only in the bronchial tree but also in the alveolar tissue. In some cases big composite nodules were observed, evidently resulting from a fusion of a number of single nodules. In the last stages of the experiment hyalinisation occured quite generally in these composite nodules. Fibrotic nodules were seldom observed in the alveolar tissue of group C. The accumulated cells were gradually eliminated by phagocytosis from the alveolar tissue which after a while attained a quite normal appearance. Fibrous tissue proliferation in group C was principally confined to the bronchial tree and especially to those areas where destruction of the epithelium had been observed. From this investigation the following conclusions were drawn:

(1) That piezoelectricity plays no role in the pathogenesis of silicosis.

(2) That the fibrosis caused by piezoelectric quartz and non-piezoelectric tridymite is more or less of the same degree.

(3) That the fibrous response to amorphous silica is principally limited to the bronchial tree while quartz and tridymite also affect the alveolar tissue.

(4) That amorphous material also induces fibrosis and the crystalline structure of the particulate matter is therefore not essential as an etiological factor.

EXPERIMENT II. **An investigation of the genesis and development of the silicotic nodule.**

The histological data of experiment I clearly indicates that the fibrous nodule must be investigated at a very early stage. This experiment was therefore conducted with 40 rats, each animal receiving an intratracheal injection of 1 ml. quartz suspension (25 mg. per ml. sol.), and two animals being killed every 24 hours over a period of 20 days. Imprints were made of each lung according to Gillman and Gillman (1949) and blood smears were also made for differential white cell counts, to determine the correlation, if any, between the cells participating in the cellular reaction of the lung and the leucocyte population of the blood.

Histological data: From the evidence obtained in this investigation, it was possible to differentiate five stages in the fibrogenesis of the nodule in the lungs of the animals during the first 20 days.

First stage: Within 24 hours after the administration of the quartz suspension, *a remarkable thickening of the alveolar walls accompanied by a pronounced cell infiltration and general edema,* was observed. At this stage the polymorphonuclear cells were in the majority but mononuclear cells were also encountered.

Second stage: The second stage (approximately 48 hours after injection) was characterised by widespread congestion which was confined to the smaller capillaries. The initial cell infiltration and septal thickening were more conspicuous than on the first day. The epithelium of the bronchioli already showed a hyperplastic proliferation and the secretion of mucin commenced.

Third stage: The third stage began approximately on the third or fourth day and was characterised by a waxy degeneration in those areas where the congestion had been most severe. A peculiar fibrin precipitate, with numerous accumulated cells, was encountered in some alveoli in the neighbourhood of the blood vessels. These cells apparently migrated from the blood vessels to the surrounding lung tissue.

Fourth stage: From the seventh day after the administration of the quartz suspension there was a noticeable improvement in the blood supply of the lung tissue. The fibrin-like precipitate in the alveolar spaces was changed to a network, but some of the smaller blood vessels were still blocked. It seemed likely that these blocked blood vessels of this stage with the cell accumulations around them were the nidi of future nodular "anlagen".

Fifth stage: This stage began approximately on the 12th day and lasted indefinitely, depending on the quantity of particulate matter in the lung tissue. *It must be regarded as the recovery stage* in which the injured tissues and cells were removed by phagocytic action. The alveolar walls became normal although they still retained some giant phagocytic cells (Figure 3). *The first indication of individual nodular "anlagen" was seen at the beginning of this stage in badly damaged lung areas,* and especially in the vicinity of the smaller blood vessels and around the alveolar bronchioli. The fibrous arrangement of the nodular "anlagen" did not yet show concentric structure (Figure 4), but during the later development it was noticed that some nodules attained a necrotic centre and that especially in the proliferating fibrous tissue of the nodules, blood vessels were absent.

The histology of the imprints: The identification of the cells in imprints of lung tissue was rather difficult because they were viewed on a background of a bloodfilm. In imprints of normal lung tissue, however, there were many lymphocytes and monocytes, with only a small number of macrophages and polymorphonuclear cells (Figure 5).

The immediate lung reaction to the administration of a particulate silica suspension *was not only the mobilisation of polymorphonuclear cells but also to a lesser degree of mononuclear cells and macrophages.* The polymorphonuclear cells steadily increased in numbers, reaching a maximum on the third day after injection. At this stage a marked increase in the number of mononuclear cells and macrophages were also noticeable. *These two cell types were inclined to accumulate in special loci to form cell plaques or cell nests (Figure 6).* It seemed likely that in these cell nests *there was a*

transformation of mononuclear cells into macrophages. From the 7th day general degenerative and destructive changes were observed in these macrophages. Their staining capacity deminished, their nuclei became vacuolated, while their cytoplasm became foamy and had an apparent reticular structure (Figure 7). It is generally accepted that these cell plaques are the origin of the future fibrotic nodules.

The blood leucocyte response to intratracheal injection of a particulate suspension of quartz: In this experiment 8 control animals (4 females and 4 males), of the same average age and weight as the experimental animals of the previous experiment, were used. They were all killed at the same time and white cell counts made to determine the average percentage of the different cell types in the normal blood. Leucocyte counts were also made in two of the experimental animals (male and femals) each day over a period of 20 days after the administration of the quartz suspension. The mean percentages of the leucocyte counts of the experimental animals was compared each day with the average obtained from the controls (Figure 8).

It was evident from the results that the *intratracheal administration of a quartz suspension disturbed the normal leucocyte relationship of the blood.* There was a relative decrease in the percentage of lymphocytes and an increase in the percentage of neutrophils. This relative neutrophilia was most noticeable during the first three days after the quartz injection. From the third day the percentage of lymphocytes increased steadily and reached a maximum on the 4th day after which it receded to the normal level. On the sixth day a slight neutrophilia was again noticed but the fluctuations gradually receded to the normal average percentage of cells of the control animals.

From the results obtained it was concluded:

(i) that the early development of the fibrous nodule can be defferentiated into several stages;

(ii) that the initial lung response to the administration of a quartz suspension is characterised by the immediate mobilisation of polymorphonuclear cells, which are later succeeded by mononuclear cells and macrophages;

(iii) that the cell plaques consist of mononuclear cells and macrophages and that they are scattered throughout the whole of the lung tissue and not limited to the lymph nodes;

(iv) that the blood leucocyte relationship is disturbed for a while after the intratracheal administration of a quartz suspension, but returns to normal within a fortnight.

EXPERIMENT III. **The genesis and development of fibrous nodules in testicular and muscular tissue.**

At this stage of the experimental work the investigations were switched to testicular and muscular tissue. The animals were divided into three groups, A, B and C, of ten animals each, and each group of rats was injected with suspensions of quartz, tridymite and amorphous silica respectively into the left testis and the musculature of the thigh. One rat of each group was killed at set times over a period of 200 days.

Testicular tissue: The experimental animals of *group A* received an injection of 0.25 ml. of a quartz suspension (50 mg./ml. sol.) in the left testis and an equal dose in the right thigh. Within three days after the injection into the testis it was noticed *that the spermatogenic epithelium had disappeared and that edema occurred.* The tubuli seminiferi contorti were blocked by cell debris and the infiltration of polymorphonuclear cells had started. The proliferation of the interstitial tissue was well on its way and a pronounced thickening of the tunica albuginea could be observed (Compare Figures 9 and 10).

After 15 days the fibrous tissue proliferation was very pronounced, involving almost the whole of the testis. In a number of tubuli a red staining fibrin-like precipitate was encountered which appeared to be reticular in some preparations but homogeneous in others (Figure 11). Single nodules, similar to those of the lungs were not found in the testis. This difference may be ascribed to the more compact nature of the testicular tissue, and apparently there was a fusion of single nodules to form one composite nodule, at a very early stage.

In the later stages the testicular tissue appeared as one solid mass of fibrous tissue in which different points of hyalinisation could be distinguished.

Group B: Each rat in this group was injected with 0.25 ml. of a tridymite suspension (50 mg./ml. sol.) in the testis and in the musculature of the thigh. The immediate tissue reaction to this suspension was *one of degeneration especially of the tubuli and interstitial tissue.* In some areas of the testis this destruction was only temporary while in others it seemed to be permanent. The tissue degeneration was followed by an infiltration of polymorphonuclear cells which accumulated in cell masses in the severely damaged areas. These cell masses were eventually encapsulated by proliferation of fibrous tissue of the adjoining areas. The subsequent development of the fibrous tissue was similar to that of group A.

Group C: The animals in this group were injected with *an amorphous silica suspension* (0.25 ml.) in the left testis and in the musculature of the thigh. The immediate testis tissue response was comparable with that observed in group B, but the destruction of the tissue elements in direct contact with the material was more pronounced. These totally destroyed areas (Figure 12) were encapsulated by progressive fibrous tissue proliferation from the less damaged areas. The contents of this fibrous capsule consisted mainly of an amorphous material and tissue debris which became infiltrated by large numbers of polymorphonuclear and mononuclear cells. *Resorption of the encapsulated masses occurred quite frequently* with the result that the testes of some experimental animals disappeared altogether from the scrotum.

Muscular tissue: The response of muscular tissue to quartz and tridymite suspensions were largely identical. For instance, an extraordinary proliferation of fibrous elements of the muscle, with degeneration of the muscle fibres, was observed (Figure 13). There were no individual nodules in the quartz and tridymite groups, but as in the case of the testis, one big fibrous mass was formed in which hyalinisation commenced in scattered loci.

As far as the animals of group C were concerned, destruction of the muscular tissue occured wherever it came into contact with the amorphous

silica. These destroyed tissue areas were eventually encapsulated by proliferation of fibrous tissue.

In this investigation the following observations were made:

(1) Quartz, tridymite and amorphous silica evoke fibrogenesis in testicular and muscular tissue.

(2) These silica derivatives evoke fibrous responses which differ from one another only in degree.

(3) Amorphous silica seems to be very toxic and causes great tissue destruction.

(4) Since no concentric nodules were observed in testicular and muscular tissue after administration of particulate matter it is concluded that the concentric arrangement of these structures in the lung is due to the specific anatomic structure of this organ.

EXPERIMENT IV. **The influence of protective substances on the genesis and development of the silicotic nodule.**

This experiment was carried out on 50 experimental animals divided into five groups as shown in Table I. Suspensions of carbon and quartz, of aluminium oxide and quartz, as well as individual suspensions of carbon and aluminium oxide were separately administered by intratracheal injections to each group of animals respectively. The animals were killed over a period of 27 days and histological preparations of the lungs were made.

Group A: These animals were injected with a quartz suspension and a typical lung response was observed as described on page 132. For comparison the principal results are tabulated here again:

(1) Thickening of the alveolar walls.

(2) Polymorphonuclear succeeded by mononuclear cell infiltration.

(3) Exudation of fluid and a general condition of edema.

(4) Congestion.

(5) The stage of repair, improvement of blood circulation and phagocytosis of tissue debris and particulate matter.

(6) Proliferation of fibrous tissue and genesis of nodules.

Group B: The animals of group B were injected with *a quartz + aluminium suspension.* One rat was killed immediately to serve as a control (Figure 14). The lung reaction to the quartz + aluminium suspension was quite different from that to quartz. *The cell infiltration and thickening of the alveolar walls were delayed for 24 hours.* A condition of edema was confined only to those areas where flocculated material had been deposited. Cell infiltration on the second and third days was minimal except in those areas where tissue destruction occured. No degeneration of the bronchial epithelium was observed. On approximately the seventh day the cell infiltration was pronounced in areas where the material was deposited. In these areas there was apparently a violent tissue reaction, *but fibrous tissue proliferation was limited and collagenous fibres were not formed.* (Figure 15.)

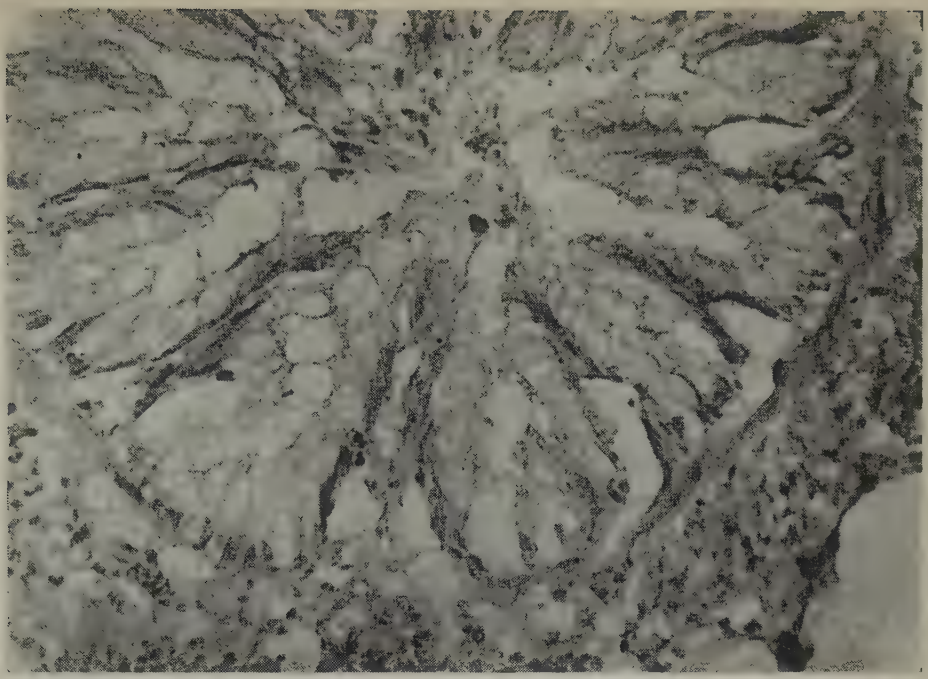

Fig. 1.
Photomicrograph showing the hypertrophied bronchial epithelium 16 days after injection of a suspension of particulate tridymite. (X 450).

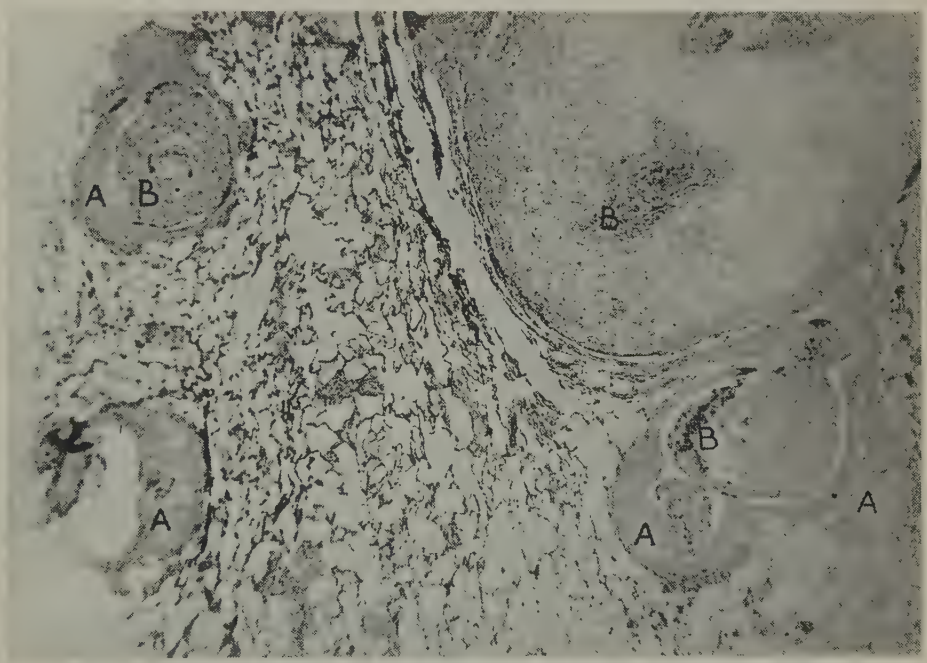

Fig. 2
Photomicrograph showing the destruction of the bronchial epithelium and connective tissue proliferation 80 days after injection of a suspension of amorphous silica. A, proliferating connective tissue; B, plugs of polynuclear and mononuclear cells. (X 100).

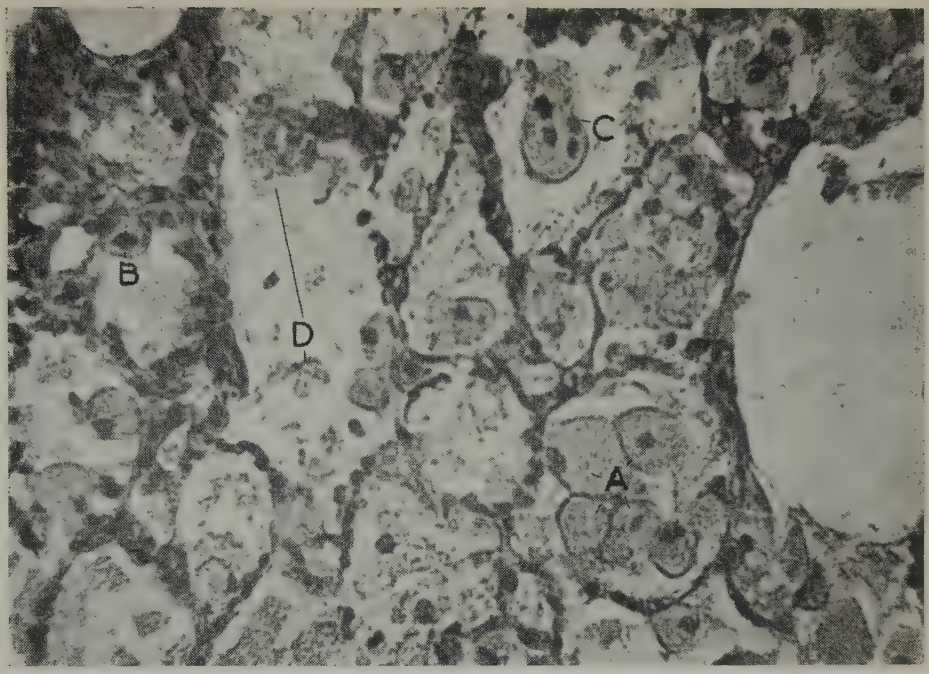

Fig. 3.

Photomicrograph showing large macrophages (septal cells) in the alveolar spaces. A, vacuolated septal cell; B, non-vacuolated septal cell; C, giant cell; D, tissue debris. (X 970).

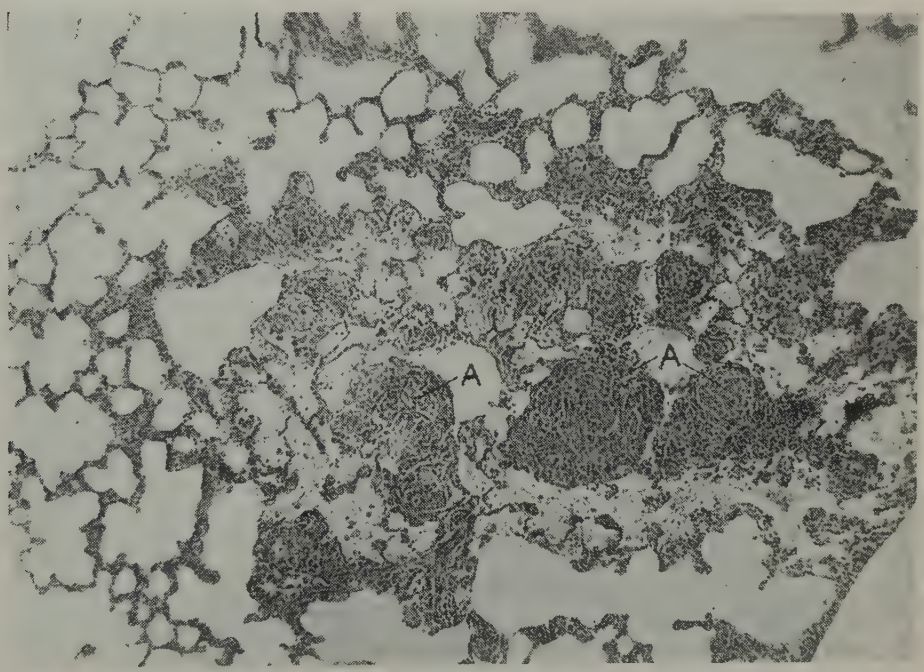

Fig. 4.

Photomicrograph to show the first nodular "anlagen" 11 days after injection of a quartz suspension. A, cell nests where nodules develop. (X 100).

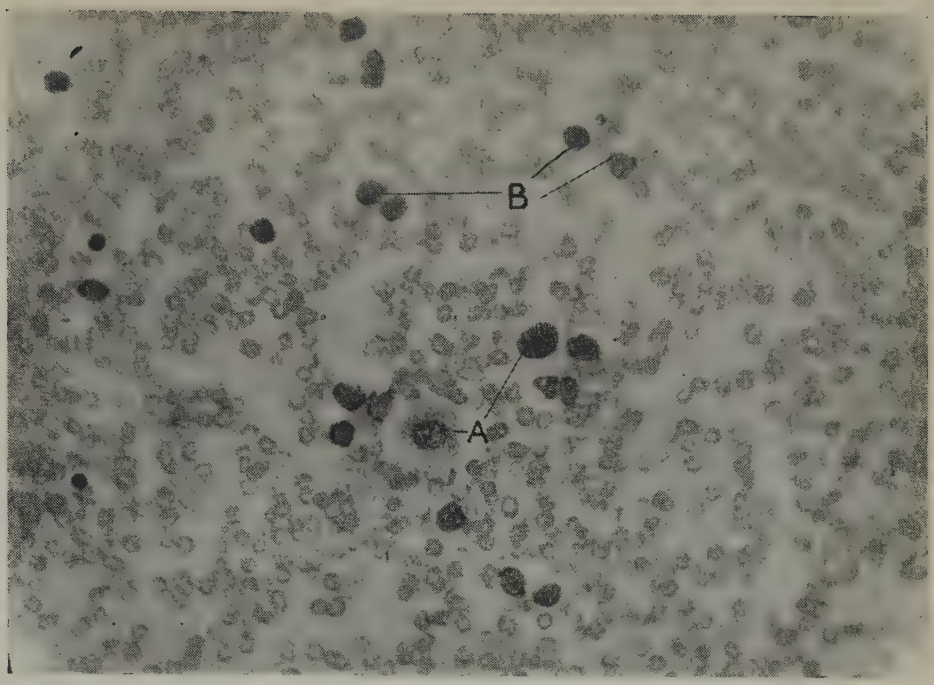

Fig. 5.

Photomicrograph of an imprint of a normal rat lung to show the distribution of different cells. A, macrophages; B, lymphocytes. In the background the red blood cells are seen. (X 450).

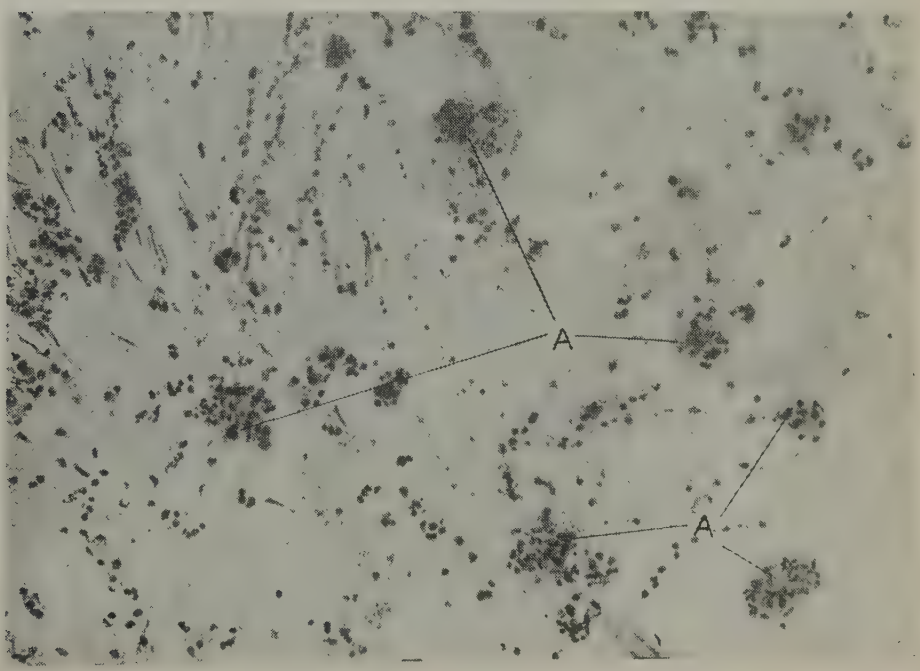

Fig. 6.

Photomicrograph of an imprint of the lung 15 days after the injection of a suspension of quartz. A, cell nests of mononuclear cells and macrophages. (X 100).

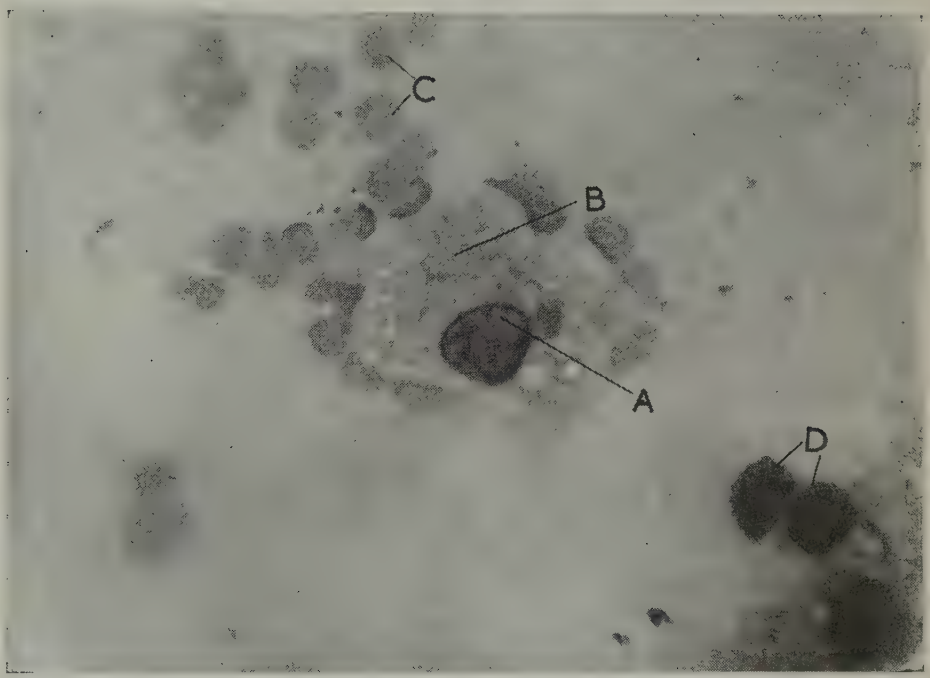

Fig. 7.

Photomicrograph to show a macrophage 7 days after injection of a quartz suspension with a vacuolated nucleus and foamy cytoplasm on the point of degeneration. A, vacuolated nucleus; B, foamy cytoplasm; C, red blood cells; D, nuclei of macrophages. (X 970).

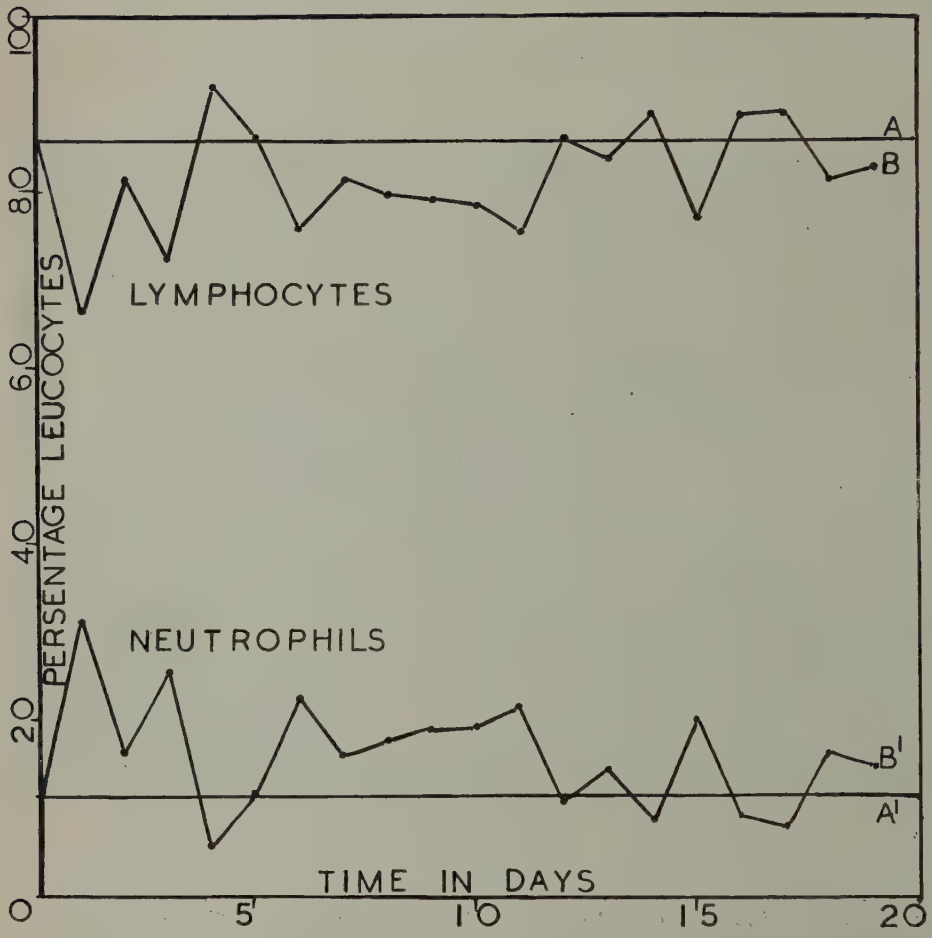

Fig. 8.

A graph to illustrate the correlation between the average percentage of lymphocytes and neutrophils of the experimental animals and that of a control group. A and A^1, average percentage of lymphocytes and neutrophils respectively, of a control group of 8 rats (4 males and 4 females) before injection of a quartz suspension.

B and B^1, average percentage of lymphocytes and neutrophils respectively, of two experimental animals (male and female), showing daily average over a period of 19 days after a single injection of a quartz suspension.

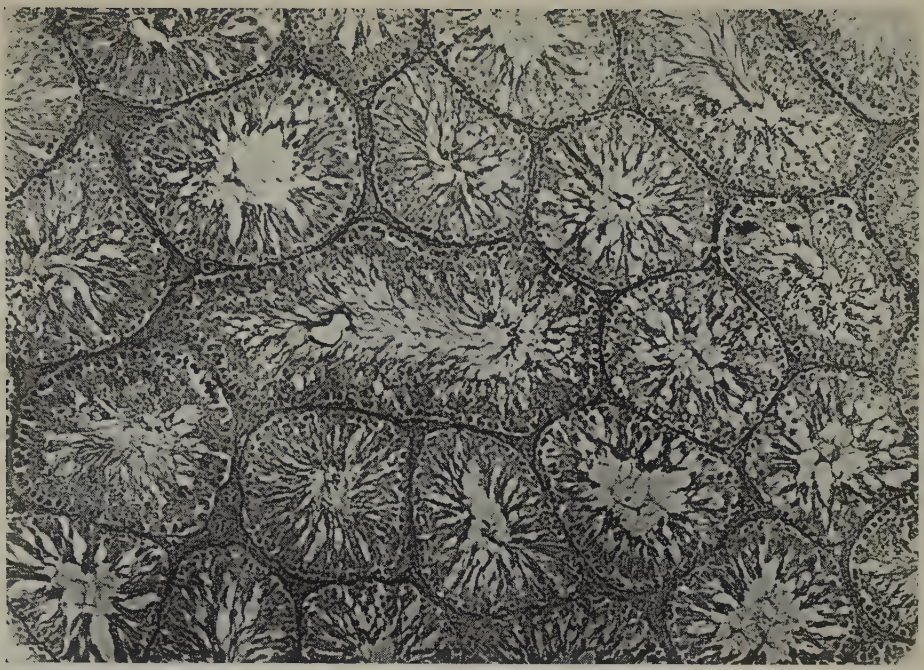

Fig. 9.

Photomicrograph of the normal testis of the rat. (X 100).

Fig. 10.

Photomicrograph showing the destruction of the spermatogenic epithelium and cell infiltration in the testis 3 days after the injection of a quartz suspension. (X 100).

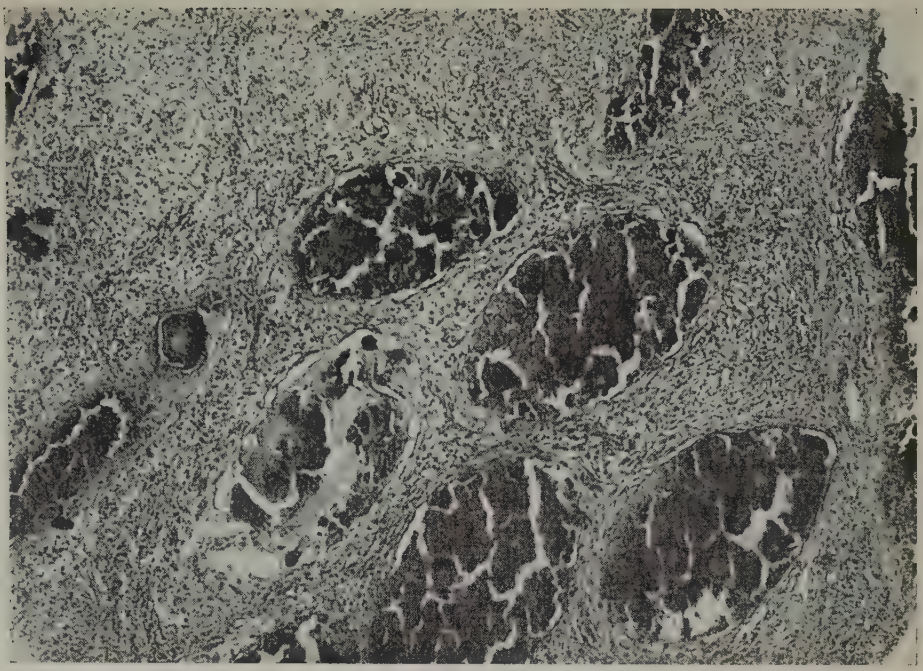

Fig. 11.

Photomicrograph showing connective tissue proliferation 15 days after injection of a quartz suspension in the testis. In some of the tubuli a fibrin-like precipitate is noticed. (X 100).

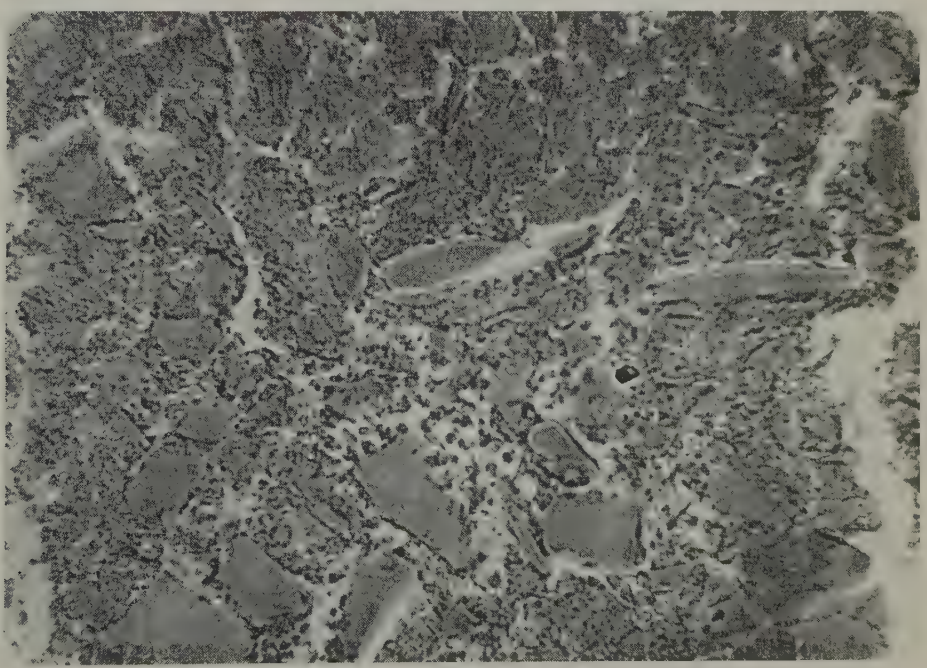

Fig. 12.

Photomicrograph showing a totally destroyed area in the testis 3 days after the injection of a suspension of amorphous silica. (X 100).

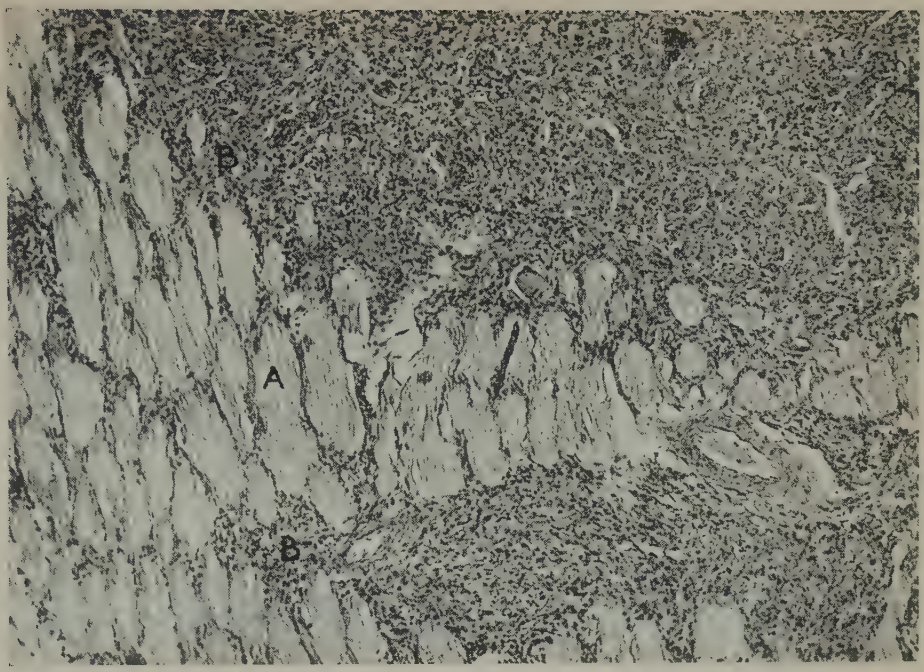

Fig. 13.

Photomicrograph showing degeneration of muscular tissue and proliferation of connective tissue. A, muscular tissue; B, connective tissue. (X 100).

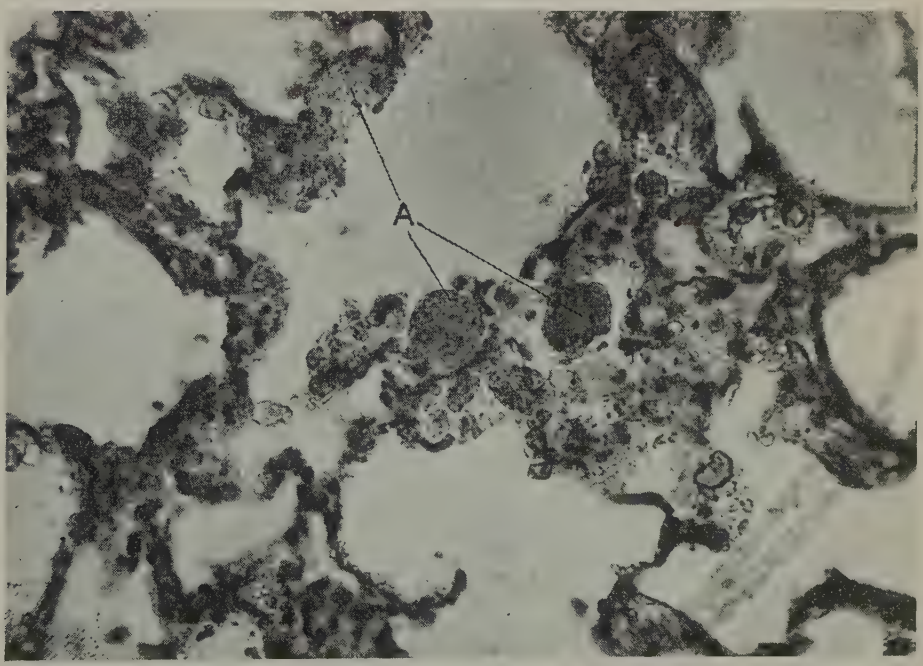

Fig. 14.

Photomicrograph to show the flocculation of a suspension of quartz and aluminium oxide immediately after injection into the rat lung. A, flocculated material in the alveolar spaces. (X 450).

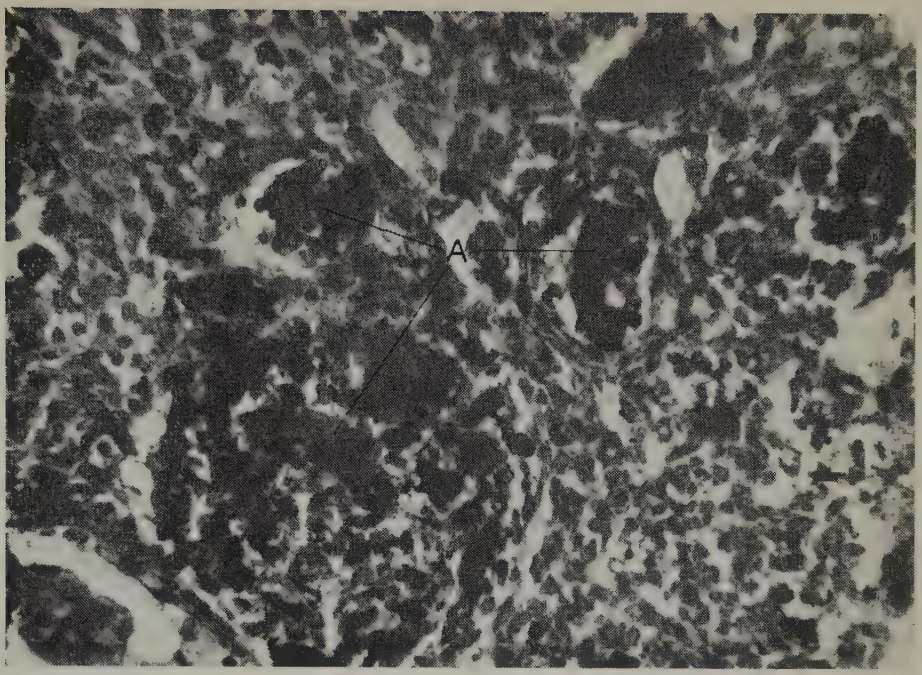

Fig. 15.

Photomicrograph to show pseudo-nodules in the lung of a rat injected with a suspension of quartz and carbon. A, pseudo-nodules surrounded by macrophages. (X 450).

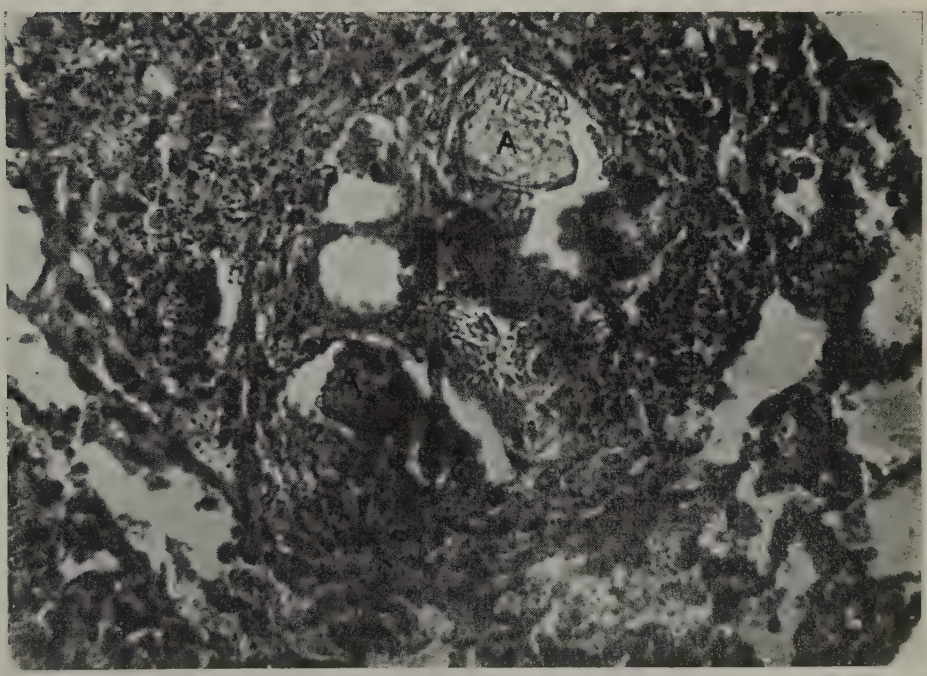

Fig. 16.

Photomicrograph to show a pseudo-nodule and flocculated material in the rat lung 25 days after injection of a suspension of quartz and aluminium oxide. A, flocculated material. (X 450).

TABLE I.

Group	No. of rats	Dose	Suspension	Ratio	Days on which rats were killed after injection.
A	10	1 ml. (25 mg./ml.)	Quartz	—	1 3 5 7 9 11 13 15 17 19 21 23 25 27
B	15	,,	Quartz + $A1_2O_3$	9 — 1	1 3 5 7 9 — 13 — 17 — 21 — 25 — 27
C	15	,,	Quartz + carbon	4 — 1	1 3 5 7 9 11 13 15 17 19 21 23 25 27
D	5	,,	Aluminium	—	1 3 - 7* - — 13* — — — — — 25* —
E	5	,,	Carbon	—	1 3 - 7 - — 13 — — — — — 25 —

From these observations the conclusion may be drawn that *aluminium oxide inhibits the fibrous tissue stimulating capacity of quartz.* The exact mechanism of this inhibition is not quite clear. The flocculated material as observed in histological preparations is evidently *an innocuous non-irritating substance formed by quartz, aluminium oxide and tissue fluid.*

Group C: The animals in this group received an injection of a carbon + quartz suspension. The immediate tissue reaction to this injection was comparable with that obtained with only a suspension of quartz.

Cell infiltration, edema and congestion were generally encountered but the reaction was not very pronounced, because the repairing phase had already set in on the 7th day. The alveolar walls became thinner, and the accumulated cells gradually disappeared from the lung tissue where no particulate matter was deposited. *The flocculated material was encapsulated by cell capsules to form pseudo-nodules* (Figure 16). Although the immediate tissue response to quartz and carbon was very violent, no real fibrous nodules developed. Carbon therefore is also capable of preventing fibrous proliferation but it is not as effective as aluminium. Aluminium inhibited the typical quartz lung reaction from the beginning whereas the ability of carbon to prevent fibrogenesis was only observed in the later stages of the reaction.

Group D: The animals of this group received an intratracheal injection of *aluminium oxide* to determine the tissue reaction to aluminium oxide alone. Unfortunately the injections in some of the animals were not successful and the tissue reaction to aluminium oxide could not be investigated in the later stages. From the limited observations made, it appeared that the aluminium oxide suspensions induced no fibrotic lesions.

Group E: These animals were injected with a carbon suspension to serve as a control to the quartz + carbon group. The cell infiltration was very much retarded during the first 24 hours, but from approximately the second day a pronounced tissue reaction was obvious. Thickening of the alveolar septa, cell infiltration and exudation of fluid were general characteristics in the areas where the material was deposited. The reaction, however, was never as violent as in the case of quartz. From the experiment it appeared that the tissue reaction to quartz and carbon, when administered separately, was more or less the same, but when administered together, the carbon apparently had an inhibitory effect on the quartz reaction. A definite antagonism also exists between quartz and aluminium as far as fibrogenesis is concerned.

EXPERIMENT V. **A comparison of the relative adsorptive capacities of pneumoconiosis-producing substances.**

In these experiments an investigation was made *of the adsorptive capacities of known silicosis-producing minerals.* The observation was made that kaolin, a well known silicosis-producing substance, is used in the Scott method (1940) of extraction of chorion gonadotrophin from pregnancy urine. Other known silicosis-producing minerals were therefore also tested for their adsorptive capacities. (Table II.)

From the experimental data presented in Table II it is obvious that there is a close correlation between the known pneumoconiosis-producing

TABLE II.

Material	Composition	Adsorptive capacity
Asbestos (blue)	$NaFe(SiO_3)_2$. $FeSiO_3$	++
Asbestos (white)	$H_4Mg_3Si_2O_9$	++
Flint*	SiO_2	++
Ganister*	SiO_2	++++
Quartz	SiO_2	++++
Kaolin	$H_4Al_2Si_2O_9$	++
Olivine	Mg_2SiO_4	+
Sericite	$(HK)AlSiO_4$	++
Slate	SiO_2 + silicates	++++
Talc	$H_2Mg_3(SiO_3)_4$	+

\+ = Weak
++ = Medium
+++ = Strong
++++ = Very Strong.

* = Piezoelectric.

substances and their adsorptive capacities. Since both piezoelectric and non-piezoelectric substances are adsorptive, the piezoelectric property as such can therefore not account for fibrogenesis as proposed by Velicogna (1946).

Because of this correlation between the known pneumoconiosis-producing

TABLE III.

Material	Composition	Adsorptive capacity
Bentonite	SiO_2 + silicates	++++
Epidote	$HCa_2(Al.Fe)_3Si_3O_{13}$	+++
Glass (Pyrex)	?	—
Silica glass (Opaque)	?	—
Quartz heated to 1,400° C for 3 hours	?	—
Nepheline	$Na.Al.SiO_4$	+
Pyrophylite	$H_2Al_2(SiO_3)_4$	++++
Topaz	$Al(FOH)_2AlSiO_4$	+
Tourmaline	Al-Borosilicate	+++
Cinnabar*	HgS	++
Magnesite	$MgCO_3$	+
Wurtzite	ZnS	++
Hematite	Fe_2O_3	+

\+ = Weak
++ = Medium
+++ = Strong
++++ = Very Strong.

* = Piezoelectric

substances and their adsorptive capacities, a further experiment was carried out to determine the adsorptive capacities of other silica derivatives and non-silicaceous minerals not known to produce silicosis. (Table III.)

It was interesting to note that all the minerals containing free silica (SiO_2) had a pronounced adsorptive capacity. Pyrex glass, opaque silica glass and quartz heated to 1,400° C for 3 hours showed no adsorption. *It appeared therefore that some intramolecular change took place in the quartz molecule during heating, as a result of which its adsorptive property disappeared.* If adsorption is regarded as an etiological factor in the pathogenesis of silicosis, then these substances should not produce fibrosis. The other silica derivatives showed different degrees of adsorption, irrespective of the different elements included in the molecule. Silica-aluminium compounds also had the adsorptive property and therefore it is difficult to explain how aluminium prevents the fibrogenesis-stimulating action of quartz. Non-silicaceous substances, such as cinnabar and wurtzite, which exhibited piezoelectric properties, only showed a moderate adsorptive capacity. Two non-piezoelectric substances, magnesite and hematite, also showed moderate adsorptive capacity.

From this experiment it was concluded that:

(1) All piezoelectric substances investigated have the adsorptive property.

(2) Minerals containing free silica are strongly adsorptive while other silicaceous derivatives are less adsorptive.

(3) When quartz is heated some intramolecular change apparently takes place by which the adsorptive property is lost.

(4) Some non-silicaceous minerals also have adsorptive properties.

DISCUSSION

It is well known that silica occurs in practically every tissue of the living organism, but its specific function in the metabolism of the body is as yet not clear. It is probably a trace element which plays an important role in some physiological oxidation-reduction process during metabolism. It must, however, be regarded as a foreign substance which is accidentally consumed with our food. In this way quite large quantities of silica reach the stomach and intestine where it is exposed to acid and alkaline media, and is dissolved and absorbed into the blood stream. It probably appears in the blood as silicic acid and is also excreted in this form in the urine. *Silica which enters the body by this route has without doubt no detrimental effects.* Silicaceous dust, entering the body through the air passages is very injurious and results in permanent pathological changes. The initial tissue response is an attempt to protect the lung tissue, and the fibrous nodules must be regarded as a lung reaction of a permanent nature to prevent the further spreading of the particulate matter. The nodules may also be regarded as scar tissue comparable to scar tissue proliferation in other sites.

(a) The experimental induction of silicotic lung fibrosis.

It is difficult to induce uncomplicated silicosis in experimental animals. Mavrogordato (1918) endeavoured to simulate the natural conditions to which industrial workers are subjected, and therefore exposed his animals to high

concentrations of dust in a dust chamber. Even this method is still artificial because the silicotic condition is induced more quickly than usual. *Natural silicosis takes many years to develop before it is so pronounced that it can be clinically diagnosed.*

It seems essential that *the dust particles should reach the alveoli* before any permanent lung damage can be caused. The delay in tissue response with the "natural" and "dust chamber" methods is due to the effective prevention by the trachea, bronchi, and bronchioli of penetration of the particulate matter to the alveoli. In the Kettle and Hilton technique (1932) where the direct injection method is applied, a high concentration of particulate matter is immediately introduced into the alveoli, and fibrogenesis therefore sets in at an early stage. A comparison of the results obtained with the "dust chamber" and the "intratracheal injection" methods showed conclusively that the eventual tissue response is identical. Direct intratracheal injection is therefore the most appropriate method for experimental purposes.

(b) **The phagocytosis of foreign particulate matter in the lung.**

The only way in which the lung can eliminate foreign particulate matter from the alveoli is by means of the phagocytes and macrophages (Mavrogordato, 1918). The foreign matter is ingested by these cells, which migrate to the ductus alveolaris, whence some are eliminated along the air passages while others are removed through the lymph vessels and deposited in the lymph nodes. Normally there are very few of these macrophages in the lung tissue, but as soon as foreign substances are introduced into the lung alveoli, large numbers are mobilised (Policard, 1947). The mobilisation and accumulation of phagocytic cells can clearly be seen in the lung preparations and imprints. The lung tissue response not only consists of the mobilisation of macrophages but also of many other cell types, e.g. *lymphocytes, monocytes, eosinophils, neutrophils, histiocytes and fibroblasts.* The relationship of these different cells to fibrous tissue proliferation is not clear. According to Maximow and Bloom (1931) the monocytes and lymphocytes can be transformed by differentiation to polyblasts which again differentiate into macrophages. *My results indicate a pronounced lymphopenia in the circulating blood* immediately after the intratracheal injection of a quartz suspension. This phenomenon is difficult to explain but the lymphocytes apparently accumulate in the lung tissue and take part in the lung tissue reaction. The septal cells of the alveolar walls are also potential sources of histiocytes and it is quite often observed that these cells detach themselves from the alveolar walls and show phagocytic activity. The histiocytes differentiate to form macrophages or even polyblasts, which can be transformed to fibroblasts (Gillman and Gillman, 1949). It is also observed that some of the endothelial cells of the blood-vessels are transformed to polyblasts. Maximow and Bloom (1931) maintain that the fibroblasts of fixed connective tissue can also differentiate to polyblasts under special circumstances, but as soon as the stimuli are removed, they differentiate to fibroblasts of connective tissue. If it is accepted that endothelial cells can differentiate to polyblasts and macrophages, there can be no objection to the assumption that *the polyblasts derived from the fibroblasts of fixed connective tissue, have the same differentiation potentialites.*

As far as the bronchial epithelium is concerned, my investigations showed that no transformation to macrophages occurs. The bronchial epithelium in direct contact with the silica particles hypertrophied for a while before degeneration set in. It became clear from this investigation that the septal cells, lymphocytes, monocytes, histiocytes and polyblasts from various sources took part in the cellular response of the lung tissue and ultimately contributed to the general fibrosis of the lungs.

(c) **The nodular "anlage".**

The first phase in the lung reaction to the introduction of foreign particulate material into the alveoli is characterized by the mobilisation of macrophages from various sources to eliminate the dust particles as quickly as possible. If the total elimination of the inhaled matter is not achieved, the second phase in the lung reaction follows, viz. an attempt on the part of the lung tissue to adapt itself to the presence of the particulate matter and to limit the damage as far as possible. In doing so the "anlagen" of the future fibrous nodules are formed by the differentiation of the macrophage and polyblast plaques to fixed fibrous tissue.

The precise loci in the lung tissue where these "anlagen" originate is still a matter of controversy. Simson and Strachan (1935) as well as Mavrogordato (1922) postulated *a correlation between macrophage plaque formation and the future nodular "anlagen".* The precise topographical distribution of these cell plaques in the lung is still uncertain but it was found that *an abundant cell accumulation occurs around the smaller arteries.* It is, however, difficult to determine whether these cell plaques consist of macrophages or polyblasts and it is therefore impossible to ascertain which cell type predominantly participates in fibrogenesis. The environment of the bloodvessel, however, *is not the only site of nodular "anlagen".* In the early stages the whole of the lung is packed with cells and in this cell mass certain foci are noticed where the future "anlagen" develop. With the Kettle and Hilton technique, *the first nodular "anlagen" are observed in the connective tissue stroma of the bronchioli.* King and Belt (1938) maintain that the "anlagen" are principally localised in the lymph glands. Gardner (1932) and Simson and Strachan (1935) postulate that the nodular "anlagen" originate in the small lymph sacs of the terminal lung units. Although these assumptions cannot be rebutted, it is clear that the nodular "anlagen" in these localities cannot explain the topographical distribution of the mature nodules in the lung, a conclusion which is corroborated by observations of fibrous tissue reactions in testicular and muscular tissue. It must, however, be admitted that in natural silicosis where exposure to dust takes place over a long period and where dust penetration into the alveoli is extremely slow, the nodules might very well be localised only in the lymph glands and vessels. But in the Kettle and Hilton method, used in this investigation, this was not found to be the case.

I am quite convinced from observations of fibrous tissue reactions in testicular and muscular tissue, *that the nidus of the fibrous tissue reaction is fixed connective tissue.* The topographical distribution of the mature nodules in the lung is therefore in correlation with the distribution of fixed connective tissue stroma of the bronchi, blood-vessels, lymph-vessels and collagenous fibrils

of the alveolar septa, which tissue is in some way stimulated by particulate matter. In histological preparations of the testis it was observed that degeneration of the epithelium of the tubuli occured wherever it was in contact with the particulate matter, but at the same time a remarkable fibrous proliferation resulted from the contact with the interstitial tissue. As far as the muscular tissue was concerned, the same observation was made, i.e. degeneration of the muscular fibres with proliferation of the connective tissue.

(d) **The histology of the silicotic nodules.**

Many investigators have commented on the concentric structure of the mature nodule (Belt and King, 1938). According to them the connective tissue proliferation starts from a central nidus, and takes on a whorled arrangement. *In my investigations on testis and muscle preparations no concentric arrangement of fibrous tissue could be observed,* nor was there a central nidus. The concentric and whorled arrangement of the fibrous tissue stroma of the lung nodules must therefore be attributed to the structure of the lung tissue itself.

As previously stated, most of the nodules in the lung develop in the bronchioli, lymph glands, lymph vessels and blood vessels. The development of the nodules can be followed clearly in the bronchioli. The particulate matter causes degeneration of the epithelium but at the same time proliferation of the adjoining connective tissue. The normal arrangement of connective tissue stroma in the bronchioli is concentric, and this arrangement is maintained in the new proliferating connective tissue. In the early stages of the development of the fibrous nodules the connective tissue is loosely arranged in the centre. In the blood and lymph vessels the nodules develop in the same way.

Sometimes, however, *the first evidence of specific lesions is seen in the aggregation of dust cells* (macrophages), in the centre of which appears a round area of polyblasts. In the subsequent development a central core of dense fibrous tissue is laid down, surrounded by a comparatively narrow zone of concentric cellular fibrosis. In these nodules the central core of dense fibrous tissue very often becomes hyaline in character. The nodules in the bronchioli, lymph vessels and blood vessels differ from the others in this respect. Hyalinisation does not occur in these nodules until the lumen is filled with dense connective tissue. *It appears as if there is a very close relationship between hyalinisation and derangement of the normal blood supply,* and it is possible that the lack of blood accelerates this development.

From the above it is clear that the fibrous nodule does not only develop *from* a central nidus, but in the bronchioli, blood vessels and lymph vessels the development can be *towards* a central nidus.

(e) **Etiological factors in the pathogenesis of silicotic fibrosis.**

When the different pneumoconiosis-producing minerals are viewed superficially one is inclined to look for a common characteristic by which the specific action of these substances, to stimulate fibrous tissue proliferation, can be explained. The early conception that fibrogenesis was the result of prolonged *mechanical irritation* by the sharp particle edges of the inhaled material was held by Mavrogordato (1918). According to this assumption

there should be a relationship between the sharpness of the particle edges and the degree of fibrosis. My investigations with amorphous silica contradict this hypothesis and the results obtained are more or less in line with those of Kettle (1932), although I do not agree with his explanation of the role of the iron oxide in preventing fibrosis. It is evident that the function of the iron oxide is similar to that of the ordinary protective substances such as aluminium and carbon.

The *chemical theory* assumes that the particulate matter must be in solution in the tissue fluids before a toxic substance can be formed which causes cell destruction, necrosis, lymphstasis, a chronic inflammatory condition and finally fibrotic changes. This toxic substance must without doubt be some silica derivative like silicic acid or colloidal silica. Gye and Purdy (1922, 1924) and Gardner and Cummings (1933) demonstrated that liver cirrhosis develops after the administration of silica. Similar results were obtained in this Department with particulate quartz (Heydenrych, 1950). It is, however, noteworthy that silicic acid absorbed from the intestines has no harmful effect on the tissues. It therefore appears that the toxicity of silicic acid or colloidal silica is closely related to its concentration in a special locality. The concentration is again dependant on the solubility of the silica preparation used in the experiments. King (1947), however, could not find any parallelism between the degree of solubility and fibrosis-producing capacity of known silicosis-producing substances. A substance like "20 Ângstrom" silica, which is very soluble, does not produce silicosis, while limestone with a particularly low solubility causes considerable fibrosis. It is therefore concluded that *silicic acid,* per se *is not an etiological factor in the pathogenesis of silicotic fibrosis.*

The *infection theory* deserves no special attention because it is possible to induce silicosis in the absence of a tuberculous infection. Another interesting view was held by Jones (1933), who concluded that the sericite content determined the silicosis-producing capacity of any single mineral. Lemon and Higgins (1935) and Fallon and Banting (1935) obtained a slight fibrous tissue reaction, but Cummings (1937) reported a remarkable tissue response with sericite. From these reports the conclusion was drawn that the contradictory results were due to the acid pretreatment of one of the sericite samples. The sericite sample treated with hydrochloric acid stimulated fibrous tissue proliferation while the untreated sample was inactive. Gillchrist and Rae (1947) assumed that the acid pretreatment rendered the sericite more soluble, and more silicic acid was therefore available to induce fibrosis. This explanation, however, contradicts the results of King (1947), who found no relationship between solubility and the pneumoconiosis-producing ability of the minerals. The greater capacity of acid-treated sericite to produce silicosis can not be explained on these grounds. (See page 146.)

Velicogna (1946) approached the silicotic problem on quite new lines. He introduced *the theory* of piezoelectric induction of silicosis and thus opened a new field of investigation. It has long been known *that silicates are not the only minerals that produce pneumoconiosis,* but as long as the chemical theory was generally accepted no explanation of fibrogenesis by other minerals than silica derivatives could be given. Velicogna (1946) assumed that all particulate matter with asymmetric crystalline structure when exposed to

pressure changes (as exists in the lung during inspiration and expiration) produces small electric currents in their immediate environment. These electric currents cause changes in the protein composition of the cells which eventually leads to connective tissue proliferation. This theory, in the first instance, assumes that all pneumoconiosis-producing substances have an asymmetric crystalline structure. With this I cannot agree. My own results demonstrate *that piezoelectric substances, such as quartz, and non-piezoelectric substances, such as tridymite and amorphous silica, are all capable of inducing fibrosis.* Piezoelectricity as a common factor in the pathogenesis of pneumoconiosis can therefore not be accepted. Evans (1948), however, postulated a recrystallisation of amorphous aluminium phosphate into asymmetric crystals. Such a recrystallisation was never observed with amorphous silica. Furthermore, it is not clear why in this instance recrystallisation would occur at body temperature while in nature much higher temperatures are neccesary for such transformation.

The present experiments *reveal no relationship between piezoelectricity and the ability of a mineral to produce pneumoconiosis.* In other words, it is not essential that pneumoconiosis-producing substances should exhibit the piezoelectric phenomenon.

I also disagree with the supersonic wave theory of Permeggiani (1947). The electrical potentials in the body are not of a kind that could make piezoelectric crystals vibrate and send out supersonic waves. The same criticism applies to Evans' (1948) assumption that piezoelectric crystals vibrate with a critical frequency which causes mechanical destruction of the tissues. This theory must therefore also be discarded.

Policard (1946) believed that more than one etiological factor was concerned in fibrogenesis, viz.:

(1) trauma induced by crystal particles;

(2) solution of the particulate matter and their chemical effect on the tissue;

(3) exchange of ions between mineral praticles and the tissue fluid.

The only contribution of Policard (1946) is his hypothesis of an ion exchange between the cations (Na, K and Ca) of the intramolecular spaces of the silica crystal and the surrounding tissue fluid. According to Policard this ion exchange process produces an alkaline medium which has an enhancing influence on fibrosis. It is, however, difficult to understand how an alkaline medium could exist while at the same time the particulate material dissolved to form silicic acid.

If this ion exchange hypothesis of Policard is accepted then it follows that the pneumoconiosis-producing capacity of a mineral is related to its ability to exchange basic ions. Quartz, a very powerful fibrous tissue stimulant, has no exchangeable basic ions, *because the intramolecular spaces are too small for ion inclusions,* and therefore should not produce fibrosis. (Quartz has a very constant refractive index which is a further proof that there are no ion conclusions in its intramolecular spaces.) The felspar minerals only produce a slight fibrosis and it is therefore expected that the exchange of basic ions must be limited, but just the opposite is found when felspar is examined.

THE ADSORPTION HYPOTHESIS

From the present investigations, especially from the results obtained with the adsorption experiments (page 138), it is clear that *the phenomenon of adsorption plays an essential role in the pathogenesis of fibrosis.* It also explains the reactions of the protective substances. This hypothesis maintains that any substance producing pneumoconiosis should show adsorption towards some hypothetical blood constituent, possibly of the nature of a polypeptide; or that the mineral particles (colloidal) are able to attach themselves (be adsorbed) to the surface of the cells. It is assumed that the hypothetical blood constituent is concentrated on the surface of the particulate matter (adsorbed) to such an extent that the surrounding connective tissue is stimulated to proliferate. Different possibilities exist as far as the nature of this adsorbed constituent is concerned. Waldschmidt-Leitz (1930) has shown that kaolin is able to adsorb an activator (glutathione) for the cathepsin enzyme system from a tissue extract. According to Baker (1929), glutathione in combination with haemoglobin forms a connective-tissue-stimulating compound. Arey (1936) on the other hand concludes that, by the action of leucocyte ferments on tissue debris and coagulated fibrin, a growth-stimulating polypeptide is formed. It is suggested that a polypeptide from the blood or tissue fluid may be adsorbed on the particulate surfaces in the localities where the particles have been deposited in the tissues, and that its growth-stimulating properties subsequently produce connective tissue proliferation.

Alternatively it is suggested that the pneumoconiosis-producing particulate matter does not adsorb a special substance, but that the particles themselves are attached to the cell surfaces by means of an ion exchange mechanism (Massart, 1949). The proteins and especially the lipoproteins of the plasma membrane of the cell are evidently very important in this process, because adsorption of the particulate (colloidal) matter to these substances will subsequently alter the permeability of the cell membrane. Any interference with the permeability of the cell membrane may have a direct influence on the oxidation-reduction enzyme systems of the cells and a local condition of anoxia may be produced. *This local anoxia may be the primary cause of the connective tissue proliferation* analogous to the influence of anoxia on the haemopoitic function of the red marrow of the bone.

The mechanism of adsorption was, until recently, not well understood but it is now assumed that this process is achieved by physico-chemical forces. The adsorption of any particulate matter (colloidal) to the cell surface is in the first instance based on the difference in electrical charge. Massart (1949) maintains that an ion exchange adsorption could only take place when the substance which must be adsorbed consists of a giant cation and a small anion or a giant anion and a small cation. A vast number of cation exchangers normally occur on the cell surface, of which the amino acid groups of the proteins, nucleic acid and lipoproteins are the most common.

It is very interesting to recall the results with acid washed sericite and ordinary sericite. Gilchrist and Rae (1947) suggest that the acid treatment makes the sericite more soluble and thus more pathogenous. Probably the sericite material is activated by the acid treatment. Even kaolin must be treated with acid before it can be used as an adsorbent in the

extraction of chorion gonadotrophin. Whether only a reversal of the electrical charge (sign) is achieved in the acid medium, or whether any other change in the composition of the particulate matter occurs, is not established, but it is well known that silica is electropositive and electronegative in acid and alkaline media respectively.

The way in which carbon and aluminium inhibit the tissue reaction of quartz can be explained by the adsorption hypothesis. It has been shown (Mavrogordato, 1922) that when coal and flint are simultaneously administered to experimental animals, coal has a beneficial influence on the phagocytosis of flint particles. Denny, Robson and Irvin (1937), on the other hand, suggest that the protective substances inhibit the fibrous tissue reaction by preventing solution of the silica particles, or by flocculation of the dissolved silicic acid. This, however, does not explain why these substances only inhibit the tissue reaction when administered simultaneously with quartz and not when given a few hours later.

Massart (1949) has proved that basic chemotherapy inhibits the respiration of yeast cells and that this inhibition can be prevented by certain concentrations of non-toxic cations of Mg^{++} and Na^{+}. In my opinion the protective substances, such as Al, Fe and C have a similar action on the pneumoconiosis-producing particulate matter. The action of these substances can be ascribed to their positive and negative charges (according to the medium) which enable them to compete with the pneumoconiotic substances for the negative or positive cation or anion exchangers of the cell surface which function as receptors. If the silica first comes into contact with the cell receptors, it is adsorbed and eventually forms an obstruction to the further attachment of protective substances. When the cell surface comes into contact with pneumoconiosis-producing substances and protective substances at the same time, the latter substances, because of their smaller molecules, are preferentially adsorbed and so again avoid pneumoconiotic substances from being adsorbed.

The effect of the protective substances must also be considered from another point of view. It is possible that a reaction may occur between the protective substances and the pneumoconiosis-producing materials in the tissue where they are deposited. Hounan (1952) has shown that the pneumoconiosis-producing dusts (negative charge) suspended in water are flocculated by the addition of non-toxic dusts (positive charge), such as aluminium sulphate or aluminium powder. From this it is evident that an ion exchange between a protective substance and a pneumoconiosis-producing substance in the tissue fluid may take place, by which the dissolved pneumoconiotic substance is flocculated and thus rendered harmless.

SUMMARY

The present study was undertaken to test some of the hypotheses concerning the etiology of pneumoconiosis which have been advanced in recent years. In order to establish the validity of the intratracheal injection method for the experimental induction of silicotic fibrosis, a comparative study of this and the dust chamber method was undertaken. Different forms of

particulate silica, such as tridymite, quartz and amorphous silica, were used for inducing silicotic fibrosis. The genesis of the silicotic nodule was studied histologically and compared with the results obtained by the injection of silica suspensions into testis and muscle. The action of protective substances such as Al and C was also studied. A new hypothesis about the etiological factors responsible for the formation of the silicotic nodule and for the action of protective substances based on the adsorption phenomenon is advanced. From the results obtained the following conclusions are drawn:

(i) Piezoelectricity and pyroelectricity cannot be regarded as etiological factors in the pathogenesis of the silicotic nodule.

(ii) A specific crystalline structure of the particulate matter is not essential for the induction of pneumoconiosis.

(iii) Silicotic nodules develop in any organ containing a fixed fibrous tissue stroma, and nodular distribution is dependent on the intimate structure of such tissue.

(iv) No direct correlation can be established between alterations in the blood picture and the cells participating in nodular development.

(v) Aluminium is a better protective substance against silicotic fibrosis than carbon.

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Contents

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ANNALS OF THE UNIVERSITY
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Volume 30, Section A, No. 3 (1954)

CONTRIBUTIONS TO THE CRANIAL MORPHOLOGY OF AMBYSTOMA MACRODACTYLUM BAIRD

by

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With 9 Text-figures

Submitted: April, 1954

ABSTRACT

A detailed description of the cranial anatomy of *Ambystoma macrodactylum* is given. The most interesting feature of the skull is the presence of two definite fenestrae basicraniales posteriores separated by a portion of the basal plate containing traces of the notochord. These are interpreted as neotenic features. In one of the specimens examined the vena capitis lateralis of one side passes through a foramen between the stylus and the foot-plate of the columella. The lacrimal is probably fused with the prefrontal. The skull is monimostylic.

CONTENTS

INTRODUCTION

The family *Ambystomidae,* according to the most recently accepted classifications, includes only three genera: *Ambystoma, Dicamptodon* and *Rhyacotriton.* In 1838 Tschudi instituted the genus *Ambystoma. Ambystoma macrodactylum,* commonly known as the long-toed salamander, was first described by Baird in 1849, but later, in 1889, Cope altered its name to *Amblystoma macrodactylum.* In 1917 Stejneger and Barbour changed the generic name back to *Ambystoma* and introduced a new specific name, *epixanthum.* According to Slevin (1928) it appears that Grinnel and Camp in 1917 re-established the specific name *macrodactylum.*

The long-toed salamander is usually found under the bark of fallen trees, or under rocks and stones, and the eggs are laid in ponds or in streams (Gordon, 1939 and Bishop, 1943). Its most pronounced taxonomic charateristic is the vomerine teeth situated in medial and lateral groups on the posterior edge of the bone.

In 1938 de Villiers made a detailed study of the suspensorial region of this species, and showed that Eaton's claim (1933) that the skull is streptostylic could not be substantiated.

MATERIAL AND TECHNIQUE

The material used for this investigation consisted of two adult specimens, a male and a female, collected by J. R. Slater and P. J. Putnam respectively and obtained from the Chicago Natural History Museum. They measured from snout to vent, 50.5 mm. and 63.5 mm. respectively, and only the heads were prepared for microtomy in the usual way. They were stained in toto in borax-carmine, embedded, and the microtomized sections, 15 μ thich, were counterstained in azan solution. The 63.5 mm. specimen was cross-sectioned, whereas sagittal sections were made of the 50.5 mm. specimen.

Graphic reconstructions were made by projecting drawings onto graph paper, according to the method of Pusey (1939).

OLFACTORY CAPSULE

The cartilaginous ethmoidal region is characterized by the absence of a septum nasale, its place being taken anteriorly by the cavum internasale and posteriorly by the planum and tectum internasale (Fig. 1). Similar conditions are found in *Trturus* (de Beer, 1937), *Ambystoma maculatum* (Theron, 1952), and *Onychodactylus japonicus* (Ryke, 1950). A nasal septum is present in *Siren lacertina* (Wiedersheim, 1877), and in *Salamandra maculosa* (de Beer, 1937). Ventrally the cavum internasale, housing the intermaxillary gland, is not bounded by any skeletal structures; dorsally, apart from the prenasal processes of the premaxillaries (Fig. 1), it has a small cartilaginous roof formed by the anterior portion of the tectum internasale. Laterally it is bounded by

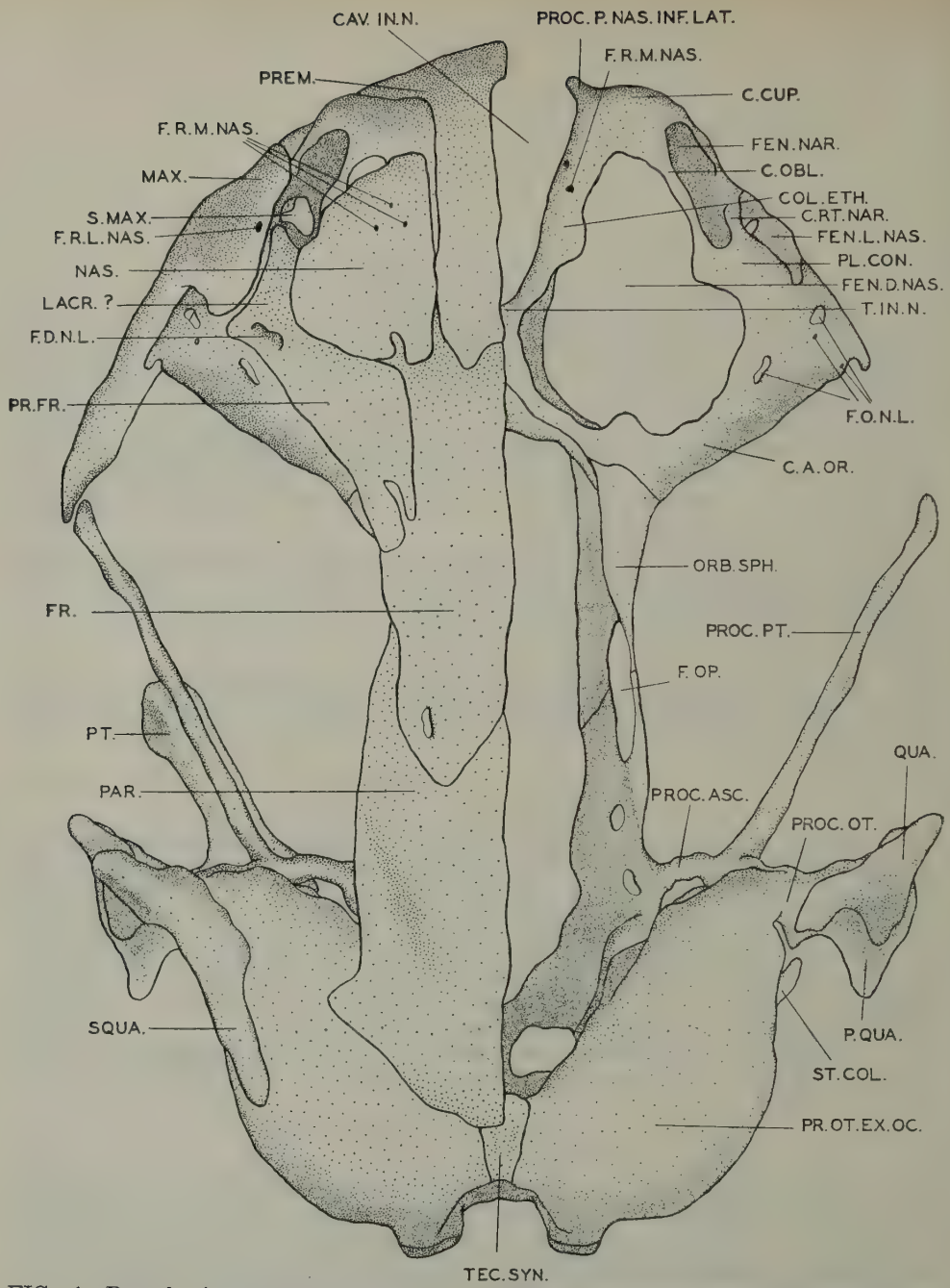

FIG. 1. Dorsal view of the skull of *Ambystoma macrodactylum*. x approx. 16.7 C.A.OR. cartilago antorbitalis; CAV.IN.N. cavum internasale; C.CUP. cartilago cupolaris; C.OBL. cartilago obliqua; COL.ETH. columella ethmoidalis; C.RT.NAR. cartilago retronarina; F.D.N.L. foramen for ductus nasolacrimalis; FEN.D.NAS. fenestra dorsalis nasi; FEN.L.NAS. fenestra lateralis nasi; FEN.NAR. fenestra narina; F.O.N.L. foramina orbitonasalia lateralia; F.OP. foramen opticum; FR. frontal; F.R.L.NAS. foramen for ramus lateralis nasi; F.R.M.NAS. foramen for ramus medialis nasi; LACR. lacrimal; MAX. maxillary; NAS. nasal; ORB.SPH. orbitosphenoid; PAR. parietal; PL.CON. planum concha'e; PREM. premaxillary; PR.FR. prefrontal; PROC.ASC. processus ascendens; PROC.OT. processus oticus; PROC.P.NAS.INF.LAT. processus praenasalis inferior lateralis; PROC.PT. processus pterygoideus; PR.OT.EX. OC. prootic-exoccipital complex; P.QUA. pars quadrata; PT. pterygoid; QUA. quadrate; S.MAX. septomaxillary; SQUA. squamosal; ST.COL. stylus columella; TEC.SYN. tectum synoticum; T.IN.N. tectum internasa'e.

the anterior medial walls of the nasal capsules, whose posterior parts are connected by the planum internasale (lamina praecerebralis). In *Megalobratrachus japonicus,* Aoyama (1930, p. 159) found a „cartilaginous plate" below the cavum internasale and „seine Spitze ist nach dorsalwärts gerichtet und zwischen der Nasenkapsel eindringend" (sic). He called it cartilago infranasale. Jarvik (1942) states that the cavum internasale of certain urodeles, such as *Amphiuma,* is incompletely divided by a median unpaired cartilago infranasalis. He regards it as a probable vestige of the internasal ridge of the *Porolepiformes.*

Born (1876) saw in the cavum internasale of the salamandrids the remains of the „Internasalraum" of the selachians. Peter (1898), however, maintains that it is only the higher urodeles which possess a cavum internasale, the lower ones having a septum. Both Peter and Wiedersheim (1877) regard the presence of the latter as primary, and the cavum internasale as a secondary arrangement caused by the development of the intermaxillary gland (Stadtmüller, 1936). Gaupp (1906) considers this as highly improbable, since in *Cryptobranchus alleghaniensis* a cavum internasale is present, although the intermaxillary gland is absent. For further information concerning the matter the reader is referred to Gaupp 1906) and Stadtmüller (1936).

A median unpaired process, the processus praenasalis superior medius, projecting forward from the tectum internasale, as in *Salamandra maculosa* (Parker, 1879), *Chioglossa* (Stadtmüller, 1936), *Onychodactylus fischerii, Hynobius* (Chung, 1931) and *Onychodactylus* (Ryke, 1950), is entirely absent in the specimens examined, as well as in *Ambystoma maculatum* (Theron, 1952) and *Diemictylus* (Chung, 1931). According to Wiedersheim (1877) *Siren* has three processûs praenasales, and he regards them as secondary outgrowths of the ethmoidal trabecular plate. Judging from Wiedersheim's figures, the two lateral processes are not homologous to the processûs praenasales inferiores laterales.

Projecting medio-anteriorly from the medio-ventral border of each dome-shaped cartilago cupolaris, is a processûs praenasalis inferior lateralis (Fig. 1), which, according to Gaupp (1906) represents the remains of the cornu trabeculae. This opinion is shared by Stadtmüller (1936), Francis (1934), and Chung (1931). The anterior part of this process abuts on the premaxillary in the angle formed by its prenasal and postnasal parts. It probably acts as a support for the premaxillary. This process appears to be generally present, but in *Diemictylus, Hynobius and Pseudosalamandra* (Chung, 1931) it is very small. Judging by Aoyama's (1930) figures this process is absent in *Megalobatrachus japonicus.*

A fenestra praecerebralis (fenestra ethmoidalis, Higgins, 1920) is absent, its place being taken by a broad planum which acts as a lamina praecerebralis. The cavum cranii and the cavum internasale therefore have no direct means of communication, a condition also found in *Ambystoma maculatum* (Theron, 1952), *Salamandra* (Francis, 1934), *Chioglossa* and *Desmognathus* (Stadtmüller, 1936). In forms such as *Onychodactylus* (Ryke, 1950), *Triturus* (Born, 1876), and *Diemictylus* (Higgins, 1920), however, a fenestra praecerebralis is present, and only connective tissue (ectomeninx) separates the cavum cranii from the cavum internasale. According to Aoyama (1930) signs of degeneration can be seen in the massive cartilago internasalis in the larval stages of *Megalobatrachus* as well as in the adult; a fenestra praecerebralis, however, is never formed.

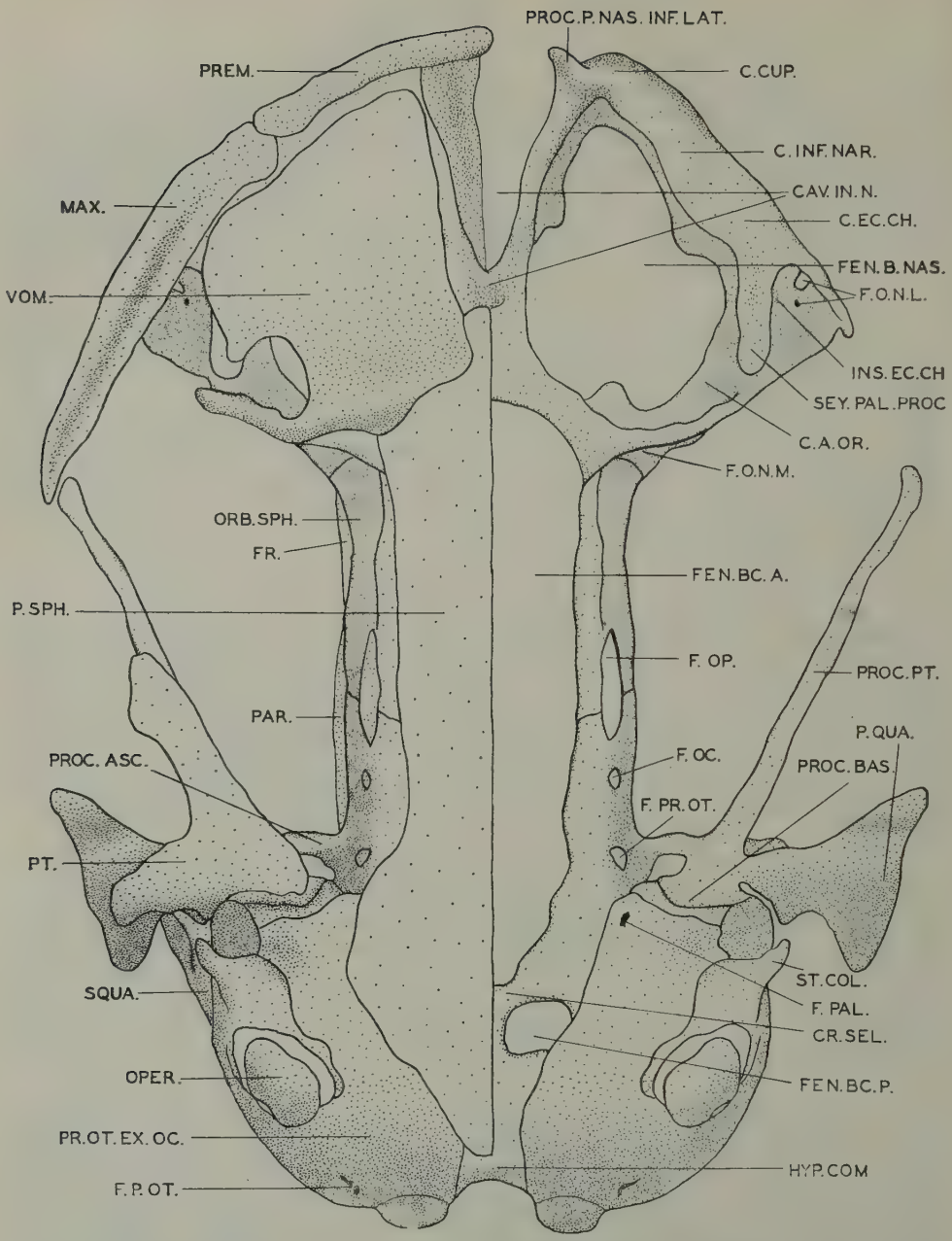

FIG. 2. Ventral view of the skull of *Ambystoma macrodactylum*. x approx. 16.7 C.A.OR. cartilago antorbitalis; CAV.IN.N. cavum internasale; C.CUP. cartilago cupolaris; C.EC.CH. cartilago ectochoanalis; C.INF. NAR. cartilago infranarina; CR.SEL. crista sellaris; FEN.BC.A. fenestra basicranialis anterior; FEN.BC.P. fenestra basicranialis posterior; FEN.B.NAS. fenestra basalis nasi; F.OC. foramen oculomotorium F.O.N.L. foramina orbitonasalia lateralia; F.O.N.M. foramen orbitonasale mediale; F.OP. foramen opticum; F.PAL. foramen palatinum; F.P. OT. foramen postoticum; F.PR.OT. foramen prooticum; FR. frontal; HYP.COM. hypochordal commissure; INS.EC.CH. incisura ectochoanalis; MAX. maxillary; OPER, operculum; ORB.SPH. orbitosphenoid; PAR. parietal; P.QUA. pars quadrata; PREM. premaxillary; PROC.ASC. processus ascendens; PROC. BAS. processus basalis; PROC.P.NAS.INF.LAT. processus praenasalis inferior lateralis; PROC. PT. processus pterygoideus; PR.OT.EX.OC. prootic-exoccipital complex; P.SPH. parasphenoid; PT. pterygoid; SEY.PAL.PROC. Seydel's palatal process; SQUA. squamosal; ST.COL. stylus columellae; VOM. vomer

A connexion between the cavum nasi and the cavum cranii is brought about by the foramen olfactorium, through which the nervus olfactorius enters the nasal capsule. It is bounded dorsally by the columella ethmoidalis and ventrally by the planum internasale.

The large, roughly triangular-shaped fenestra dorsalis nasi is bounded medially by the columella ethmoidalis, anteriorly by the cupola, anterolaterally by the lamina obliqua, postero-laterally by the planum conchale, and posteriorly by the cartilago antorbitalis (Fig. 1). The fenestra transmits bloodvessels and nerve fibres of the medial terminal branch of V (a). Higgins (1920) has shown that resorption of the cartilage (of the planum tectale) takes place during ontogeny, so that a large fenestra in the adult would be secondary, a view shared by Stadtmüller (1936) and Jarvik (1942). In *Hynobius* (Chung, 1932) the tectum nasi is more complete in the larva than in the adult. Terry (1906) mentions a 40-45 mm. *Ambystoma* possessing a continuous roof. In *Ambystoma macrodactylum* there is a small foramen for nerve fibres of the ramus medialis nasi in the cartilago antorbitalis of the left nasal capsule, presumably indicating that the resorption of the cartilage on this side has not been completed. A somewhat similar foramen was found in *Ambystoma maculatum* (Theron, 1952), on the right antero-medial edge of the fenestra dorsalis nasi.

Antero-lateral to this fenestra and separated from it by the lamina obliqua, lies the fenestra narina, which is bounded anteriorly by the cupola posteriorly by the lamina retronarina and ventrally by the lamina nariochoanalis (infranarina of Chung, 1931). It is through this fenestra that the main chamber of the nasal sac communicates with the exterior by means of the anterior external naris. Furthermore, it accommodates the muscles of the breathing mechanism and also the glandula nasalis externa. The ductus nasolacrimalis enters the fenestra through its own foramen in the septomaxillary. In *Spelerpes* (Stadtmüller, 1936) the opening of the duct into the recessus lateralis of the nasal cavity is separated from the apertura nasalis by a cartilaginous bar, thus dividing the fenestra narina into a fenestra endonarina posterior and a fenestra endonarina anterior (Jarvik, 1942).

According to Higgins (1920) the cupola, formed from pro-cartilage cells dorsal to the anterior end of the olfactory sac, is responsible for the change in position of the naris from a terminal to a lateral position.

The fenestra lateralis nasi, separated from the fenestra narina by the lamina retronarina, houses the lateral bulge of the nasal sac, or the so-called Jacobson's organ (Jarvik, 1942). The fenestra is bounded medially by the planum conchale and ventrally by the lamina ectochoanalis. It transmits the ramus lateralis nasi, which then continues through a foramen in the pars facialis of the maxillary to supply the skin of the lateral part of the snout. Similar conditions obtain in some specimens of *Ambystoma maculatum;* in others the nerve follows a different course to its destination in the skin (Theron, 1952). A similar fenestra lateralis nasi is present in *Diemictylus, Hynobius retardatus, Hynobius naevis, Onychodactylus japonicus* (Chung, 1931) and in *Ambystoma maculatum* (Theron, 1952). But in *Pseudosalamandra, Hynobius leechii* (Chung. 1931) and in a specimen of *Onychodactylus japonicus* examined by Ryke (1950), it communicates with the incisura ectochoanalis of Chung (1931). Conditions in *Onychodactylus japonicus* thus show that the relationships of this fenestra may vary even within a species. In *Onychodactylus fischerii* and in *Hynobius*

nebulosus the fenestra is absent (Chung, 1931). Of the fenestra lateralis nasi of *Hynobius leechii* Chung (1932, p. 348) writes: "Bezüglich dieser Region befindet sich Hynobius leechii daher in einem viel primitiveren Zustand als die anderen Urodelen." According to Jarvik (1942) the fenestra lateralis nasi is an entirely new formation caused by the lateral bulge of the nasal sac.

Ventrally, in the solum nasi, lies the large fenestra basalis nasi or fenestra endochoanalis (Jarvik, 1942). It is bounded anteriorly by the cupola, anterolaterally by the lamina infranarina, and laterally by the lamina ectochoanalis or Seydel's palatal process (Gaumenfortsatz, Seydel, 1895), which is merely a backward continuation of the cupola (Fig. 2). The trabecula forms the medial wall. The incisura ectochoanalis separates the palatal process from the lateral wall of the nasal capsule and is in some cases confluent with the fenestra lateralis nasi, as in *Pseudosalamandra* (Chung, 1931). The choana is situated in the posterior portion of the fenestra basalis nasi.

The cartilago obliqua, separating the fenestra narina from the fenestra lateralis nasi, is continued backward as the planum conchale (Fig. 1). In the anterior portion of the latter is a groove or indentation, the so-called impressio conchalis or external nasolacrimal groove of Jarvik (1942). It lodges the naso-lacrimal duct and part of the glandula nasalis externa. According to Jarvik (1942) the groove is a secondary formation, and according to de Beer (1937) it represents an exceedingly rudimentary concha nasalis. Both the planum conchale and the impressio conchale are entirely absent in *Ambystoma tigrinum* (Grote, 1926). The crista rostrocaudalis, which descends from the ventral surface of the planum conchale and forms a medio-dorsal support for that part of the recessus lateralis known as Jacobson's organ, is so well developed that it fuses with the dorsal surface of the solum nasi (Fig. 3). Jacobson's organ is therefore not a deep grove at the side of the main nasal cavity, but is cylindrical and is separated from the main nasal cavity not only by its epithelium, but also by the crista rostrocaudalis. Similar conditions obtain in *Ambystoma maculatum* (Theron, 1952).

Posteriorly the planum conchale passes over into the cartilago antorbitalis, which separates the nasal cavity from the orbit (Fig. 1). In the broad cartilago antorbitalis are a number of foramina, the orbitonasal foramina, which, according to de Beer (1937), indicate the boundary line between the lamina orbitonasalis and the preoptic root of the orbital cartilage. Terry (1906), describing the nasal skeleton of *Ambystoma maculatum* maintains that the antorbital process is an independent chondrification, whereas Higgins (1920), working on the same species, states that it arises from the trabecula. Lateral to the foramen olfactorium and perforating the medial part of the cartilago antorbitalis, lies the large foramen orbitonasale mediale (Fig. 2) for transmission of the ramus medialis nasi and the ramus ventralis nasi. Numerous tubules of the glandula nasalis interna are lodged in the foramen. The ramus medialis nasi accompanied by a bloodvessel enters through the dorsal part of the foramen, whereas the ramus ventralis nasi enters ventrally, anterior to the musculus obliquus inferior. It passes ventrally to join the palatine ganglion of the ramus palatinus, from which nerve fibres of the combined nerve continue forward mesially to the choana and dorsally to the vomer. The courses of the rami are similar to those in *Salamandra* as described by Francis (1934).

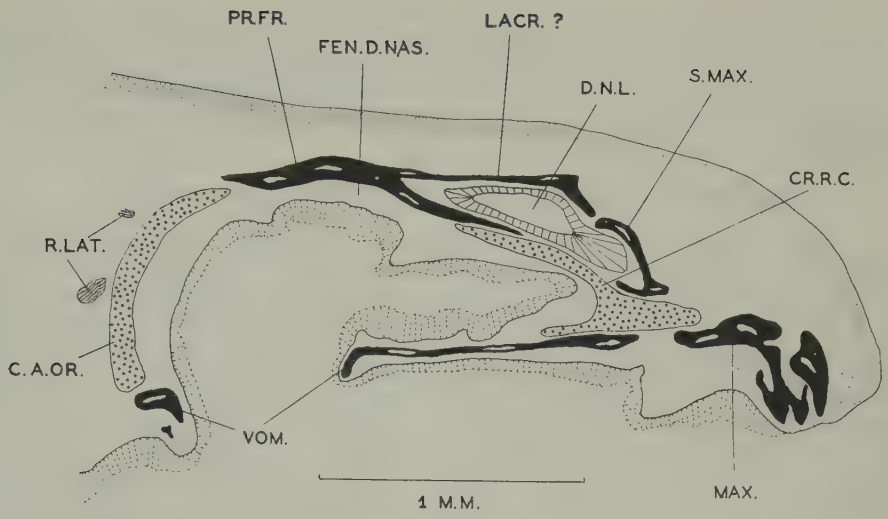

FIG. 3. Sagittal section through the nasal capsule.
C.A.OR. cartilago antorbitalis; CR.R.C. crista rostrocaudalis; D.N.L. ductus nasolacrimalis; FEN.D.NAS. fenestra dorsalis nasi; LACR. lacrimal; MAX. maxillary; PR.FR. prefrontal; R.LAT. ramus lateralis nasi; S.MAX. septomaxillary; VOM. vomer.

The determination of the homology of the foramina orbitonasalia lateralia is somewhat difficult, for not only do the foramina vary in the different species, but they may also be different on the two sides of the skull of an individual specimen. So, for instance, four foramina occur in the right olfactory capsule and three in the left capsule of one of the specimens examined. On the right side the ramus lateralis nasi and the arteria orbitonasalis each has its own foramen, the nerve running medially to the artery. On the left side of the capsule, however, the ramus lateralis nasi and the artery go through the same foramen. The remaining smaller foramina of both sides give passage to vasa orbitonasalia. In both *Triturus* (Stadtmüller, 1936) and *Diemictylus* (Chung, 1931) there is a single foramen on either side, whereas in *Ambystoma maculatum* (Theron, 1952) there are three.

According to Jarvik (1942) the term foramen apicale has been applied by various authors to those openings in the roof of the nasal capsule through which the ramus medialis nasi leaves the nasal cavity, thus accounting for the ventral foramen apicale of Stadtmüller (1936) and the dorsal foramen of Chung (1931). Again the number of foramina present on either side of the nasal capsule differs in the specimens examined. On the left side there are three foramina in the lamina ethmoidalis (columella ethmoidalis) posterior to the processus praenasalis inferior lateralis. Through the middle foramen the ramus medialis nasi leaves the nasal capsule; through the smaller posterior one passes a branch of the ramus medialis nasi, whereas the anterior foramen transmits a bloodvessel. On the right side the posterior foramen is absent; the ramus medialis nasi in this case passes through the two remaining foramina, whereas the bloodvessel again passes through the anterior foramen.

A slight deviation from this condition was found in the floor of the right nasal capsule. Between the lamina nariochoanalis and the lamina ectochoanalis is a small foramen, just ventral to the lateral bulge of the nasal sac. It occurs, however, only in the right nasal capsule. Perhaps it is also a new formation correlated with the lateral bulge of the nasal sac, as suggested by Jarvik (1942) for the fenestra lateralis nasi.

MEMBRANE BONES OF THE OLFACTORY REGION

The anterior portion of the upper jaw is formed by the premaxillaries, which are completely separate, a condition also found in *Onychodactylus* (Ryke, 1950), *Megalobatrachus* (Aoyama, 1930), *Ambystoma tigrinum* (Grote, 1926), and *Ambystoma maculatum* (Theron, 1952), whereas in *Triturus* (Gaupp, 1906) the bones are united. According to Stadtmüller (1936) Oscar Hertwig distinguished three portions in each premaxillary. The tongue-shaped pars praenasalis is the largest and forms, to a certain extent, a skeletal roof for the cavum internasale. It partly overlaps the anterior portion of the frontal (Fig. 1), but does not overlap the nasal as well, as in *Salamandra* (Francis, 1934). The degree of development of the pars praenasalis differs in the various urodeles, being extensive in the *Ambystomidae* and *Salamandridae,* and short in the primitive *Hynobidae,* so that Jarvik (1942) comes to the conclusion that the pars praenasalis has undergone progressive development in the *Urodela.* The pars palatina forming the bony palate is very poorly developed and is entirely absent in *Ambystoma tigrinum* (Grote, 1926). Francis (1934) found a "strong bar of bones" in *Salamandra.* As mentioned previously, the tooth-bearing pars dentalis lodges the processus praenasalis inferior lateralis. The posterior end of the premaxillary is overlapped by the maxillary.

Completing the arch of the upper jaw laterally are the maxillaries. Again three portions can be distinguished in each bone: a pars facialis, a pars dentalis and a pars palatina. The dorsal pars facialis does not overlap the prefrontal as in *Ambystoma maculatum* (Theron, 1952). It forms a roof over the fenestra lateralis nasi, and on its outer surface near its upper border there is a slight indentation, into which fits the lateral part of the musculus dilatator nasi accessorius. Posterior to this it is pierced by the ramus lateralis nasi on its way to supply the skin. The lateral bulge of the nasal sac fits snugly into the angle formed by the pars facialis and the pars palatina. The latter corresponds to the similarly named portion of the premaxillary, but is better developed; it does not, however, reach the vomer, as in *Salamandra* (Francis, 1934). The large tooth-bearing pars dentalis extends backwards as far as the orbital region. The processus posterior maxillaris (the backward extension of the pars dentalis) is not in direct contact with the processus pterygoideus, which ends a short distance posterior to it and is connected to it by means of a tenuous ligament. In *Ambystoma maculatum* (Theron, 1952) the tip of the processus pterygoideus overlies the processus posterior maxillaris.

The more or less triangular-shaped nasal practically covers the large fenestra dorsalis nasi. The posterior portion of the fenestra is covered by the prefrontal and frontal, whose anterior tips are overlapped by the nasal. Posteriorly the nasal shows a slight bifurcation, and postero-laterally it overlies the prefrontal. It does not overlie the pars praenasalis of the premaxillary as in *Salamandra* (Francis, 1934). Various fibres of the ramus medialis nasi pierce the nasal.

The only preorbital membrane bone is the prefrontal. Its anterior tip is wedged between the facial process of the maxillary and the lateral border of the nasal. It stretches from the cartilago obliqua, overlies the lateral part of the frontal, covers the postero-lateral part of the fenestra dorsalis nasi, and reaches to about the middle of the orbitosphenoid. Its shape and relations are best understood by referring to Fig. 1. Postero-medially, dorsal to the frontal, the prefrontal is bifurcated to a greater extent than the nasal. It is pierced by various small foramina. The prefrontals are absent in *Proteus, Necturus* and *Spelerpes* (Stadtmüller, 1936).

According to Gregory (1920) most of the urodeles have sacrificed a lacrimal, though it is still present in *Gyrinophilus* (Cope, 1889) and in *Ranodon* and *Ellipsoglossa* (P. & F. Sarasin, 1890). A lacrimal is also present in *Onychodactylus* (Chung, 1931 and Ryke, 1950), though de Beer (1937) states that it may become fused with the prefrontal. In *Ambystoma macrodactylum* and in *Triturus* (de Beer, 1937) the nasolacrimal duct pierces the so-called „prefrontal", a condition described by Jarvik (1942) as secondary. Judging from the position and relations of the lacrimal in *Rhipidistia* and *Labyrinthodontia* and in those urodeles possession a distinct lacrimal, it appears highly probable that in *Ambystoma macrodactylum* and in *Ambystoma maculatum* this bone is fused with the prefrontal (Fig. 3).

The septomaxillary (nariodal, Jarvik, 1942) fits into the posterior corner of the fenestra narina, lying medially to the pars facialis of the maxillary, laterally to the nasal, and anteriorly to the prefrontal (Fig. 1). The septomaxillary surrounds the ductus nasolacrimalis dorsally, laterally, medially and incompletely also ventrally (Fig. 3); it does not, however, form a complete canal as in *Onychodactylus* (Chung, 1929). Anteriorly it has a small foramen for transmission of a bloodvessel, and it also houses that part of the nasal sac into which opens the nasolacrimal duct. In addition the musculus dilatator nasi accessorius arises from the lateral portion of the bone.

Judging from the publications by Lapage (1928) and Jarvik (1942) the septomaxillary is regarded as a cartilage bone. But if this is true, it would mean that the septomaxillary of the urodeles is not homologous to that of the *Anura* e.g. *Calyptocephalus,* where it is definitely an acknowledged membrane bone (Reinbach, 1939). In both *Calyptocephalus* and in *Ambystoma macrodactylum* the septomaxillary is separated from the nasal cartilage by connective tissue. It is present in *Hynobius* (Chung, 1929), *Onychodactylus* (Ryke, 1950) and *Ambystoma maculatum* (Theron, 1952), though absent in *Salamandra* (Lapage, 1928), *Diemictylus* (Chung, 1929), *Megalobatrachus* (Aoyama, 1930) *Proteus* and *Siren* (Stadtmüller, 1936).

On the ventral side of the ethmoidal region, there are tooth-bearing, paired vomers; each of these covers the fenestra basalis nasi and to a very great extent the whole nasal capsule, though it does not articulate laterally with the maxillary and the premaxillary as in *Salamandra* (Francis, 1934) and *Megalobatrachus* (Aoyama, 1930). Antero-medially the vomers are very far apart, thus leaving the cavum internasale completely devoid of a floor (Fig. 2). In the postero-lateral border there is a pronounced incisure, which bounds the choana anteriorly, medially, and posteriorly and thus incompletely divides the vomer into a broad, flat anterior portion and a narrow, posterior, laterally-projecting, dentigerous strip. The vomerine teeth, situated along the posterior edge of the bone, are

arranged in a medial and a lateral group. In *Ambystoma maculatum* (Theron, 1952) the teeth also appear behind the choana, but in a transverse, undulating line, whereas in *Megalobatrachus* (Aoyama, 1930) they are confined to the anterior edge of the vomer. Postero-medially the vomer underlies the anterior tip of the large parasphenoid. A posteriorly directed, dorsal blade in close proximity to the parasphenoid, as described by Theron (1952) for *Ambystoma maculatum,* is not present. The condition might therefore be similar to that of *Megalobatrachus* (Aoyama, 1930), where the palatine is formed but disappears during metamorphosis; or the palatine might become completely fused with either the vomer or the pterygoid during ontogeny to form a vomeropalatine or a pterygopalatine (Stadtmüller, 1936). Either condition would account for the absence of the palatine.

ORBITO-TEMPORAL REGION

Connecting the nasal capsules with the otic capsules is a pair of vertical, medially concave lamellae. This region of the chondrocranium is completely devoid of both roof and floor (Figs. 1 and 2). The dorsal opening is larger than the ventrally situated fenestra basicranialis anterior, which is bounded anteriorly by the planum internasale and posteriorly by a narrow cartilaginous bar, the crista sellaris, which also joins the two side walls of the brain case to each other (Fig. 2).

The anterior ossification of the side wall, the orbitosphenoid, stretches from the cartilago antorbitalis to the middle of the foramen opticum. Similar conditions are found in *Onychodactylus* (Ryke, 1950) and *Ambystoma maculatum* (Theron, 1952), where the foramen opticum is also posteriorly bounded by the posterior cartilaginous extension of the side wall of the cavum cranii (Fig. 2). In *Salamandra* (Francis, 1934) and in *Megalobatrachus* (Aoyama, 1930) the foramen opticum lies entirely within the orbitosphenoid. The nervus opticus, surrounded by connective tissue, passes through the posterior portion of the foramen. Theron (1952) mentions the transmission of a bloodvessel accompanying the nervus opticus in *Ambystoma maculatum,* but it was not observed in the specimen examined. Slightly posterior to the foramen opticum and separated from it by the pila metoptica of de Beer (1937), lies the very much smaller foramen oculomotorium, which gives passage to the nervus oculomotorius and the arteria ophthalmica (Figs. 1 & 2). A separate foramen for the latter artery occurs in *Onychodactylus* (Ryke, 1950).

Enclosed between the cartilaginous lateral side wall of the brain case and the anterior dome of the otic capsule, is the foramen pro-oticum (Fig. 2). In it lies the ganglion Gasseri and through it pass the nervus trigeminus and nervus abducens, and a bloodvessel. The nervus abducens of *Salamandra* (Francis, 1934) leaves the cranial cavity by its own foramen abducentis, and in *Triturus* (Francis, 1934) and in the specimen examined, the nerve passes through the ganglion Gasseri and leaves it together with the nervus trigeminus.

The arteria carotis interna, running in a groove on the ventral side of the lateral wall of the brain case, and covered by the parasphenoid, enters the cranial cavity through the fenestra basicranialis anterior. The place of entry is marked by a slight incisure. Corresponding conditions are found in *Ambystoma maculatum* (Theron, 1952). A foramen trochleare as mentioned for *Megalobatrachus* (Aoyama, 1930) is absent, the nervus trochlearis passing through the parietal.

The sclerotic cartilage, which is well developed, surrounds the eyeball, leaving only the anterior face free. Posteriorly it has a large foramen for transmission of the nervus opticus, and both ventrally and dorsally bloodvessels and branches of the ciliary nerve pierce the cartilage. For further interesting facts about the sclerotic cartilage of the *Urodela* in general, the reader is referred to Theron's work (1952).

MEMBRANE BONES OF THE ORBITO-TEMPORAL REGION

The large, flat, paired frontals form a dermal roof over the anterior portion of the cavum cranii and posterior portion of the fenestra dorsalis nasi (Fig. 1). Anteriorly each frontal is overlapped by the pars praenasalis of the premaxillary and the nasal, and antero-laterally by the prefrontal. Posteriorly the frontal overlies the parietal to a certain extent and in this overlapping part is a foramen completely filled with connective tissue (Fig. 1).

Posteriorly the roof of the cavum cranii is formed by the extensive parietals, which reach backwards as far as the anterior portion of the otic capsules, which they overlap (Fig. 1). Antero-laterally each parietal possesses a processus orbitalis (Wiedersheim, 1877), which is contiguous to the cranial side-wall, and through which the nervus trochlearis leaves the cavum cranii to supply the musculus obliquus superior. The supratemporal crest, mentioned by Theron (1952) for *Ambystoma maculatum,* is either absent or may be represented by a small, laterally projecting protuberance. The fossa of Parker is therefore absent.

Stretching from just behind the cavum internasale to the occipital region, the large parasphenoid forms the dermal floor to the brain case. Underlying the vomers anteriorly, it covers both the fenestra basicranialis anterior and posterior (Fig. 2 & 4).

OTIC AND OCCIPITAL REGIONS

As in most *Urodela* (Stadtmüller, 1936), the exoccipital and the prootic are fused to form a prootic-exoccipital complex. Stadtmüller's term, „occipito-petrosum", for this compound bone is misleading, as the petrosum of mammals arises from at least two and possibly more than two otic elements. In *Onychodactylus* (Ryke, 1950) the two bones are fused dorso-laterally, but medially and ventrally they are separated by strips of cartilage. In *Desmognathus* and *Dicamptodon* there is a synostosis between the prootic and the parasphenoid (Stadtmüller, 1936). Dorsally the otic capsules are connected by the cartilaginous tectum synoticum. From the known facts of urodelan craniogenesis it appears that a fenestra basicranialis posterior is either never formed, e.g. *Megalobatrachus* (Aoyama, 1930), or when it is developed it has a varying fate. In *Necturus,* in which it does occur, it later becomes confluent with the fenestra basicranalis anterior through resorption of the crista sellaris (de Beer, 1937). In *Amphiuma* (Winslow, 1898) there are initially two fenestrae basicraniales posteriores, bounded in front by the crista sellaris and separated from each other by the notochord flanked by cartilage. Later in the ontogeny the crista sellaris and the notochord and its flanking cartilage break down, so that these fenestrae become confluent with the fenestra basicranialis anterior to form a fenestra basicranialis communis. In forms like *Salamandra,*

and in others in which it is apparently absent during the early stages of the ontogeny, a secondary fenestra is carved out of the basal plate through resorption of the cartilage (Stadtmüller, 1936). The degree of resorption varies: in *Onychodactylus* (Ryke, 1950) it is merely slit-like, whereas in most *Urodela* a medium sized fenestra is formed. The notochord which traverses the fenestra basicranialis posterior during the early stages of ontogeny, usually disappears later, though vestiges of it can remain, as in *Onychodactylus* (Ryke, 1950). The crista sellaris is broken down in *Diemictylus, Plethodon, Spelerpes* and *Desmognathus* (Stadtmüller, 1936), so that these forms possess a large fenestra basicranialis communis. In *Ambystoma macrodactylum* there are two definite fenestrae basicraniales posteriores (Fig. 2): a unique condition for an adult urodele. The fenestrae are separated by vestiges of the notochord incorporated in the medial portion of the basal plate (Fig. 4). The ontogeny of these fenestrae is unknown, but judging by conditions in other urodeles, it would appear that they are either of a primary nature, homologous to those occuring in the early ontogeny of *Amphiuma,* or that the condition is due to incomplete resorption of the basal plate. Whichever of these interpretations proves to be correct, the condition must in either case be due to neoteny. The fenestrae are anteriorly and posteriorly bounded by the crista sellaris and hypochordal commissure respectively.

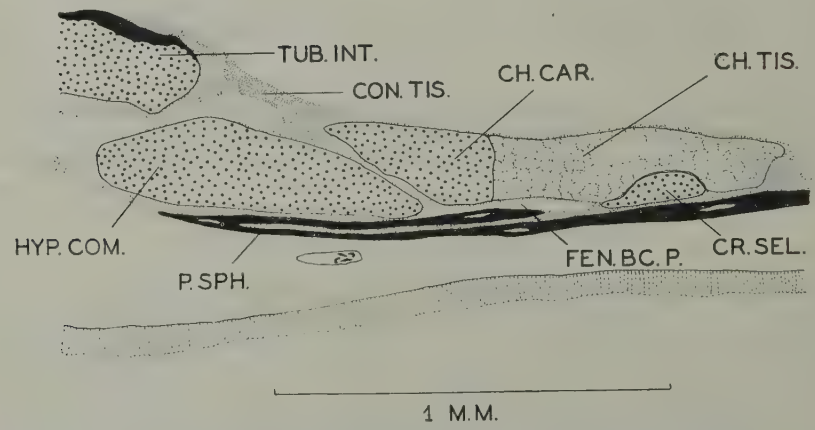

FIG. 4. Sagittal section through the basal plate and notochord. CH.CAR. chorda cartilage; CH.TIS. chorda tissue; CON.TIS. connective tissue; CR.SEL. crista sellaris; FEN.BC.P. fenestra basicranialis posterior; HYP.COM. hypochordal commissure; P.SPH. parasphenoid; TUB.INT. tuberculum interglenoidale.

Dorso-laterally, upon the disappearance of the processus oticus, there is a slight crest-like formation, formed by the crista parotica. The antero-dorsal tip of the squamosal lies immediately ventral to it, possibly "serving as a fulcrum for its movement" (Eaton, 1933, p. 523). This statement will later be discussed in more detail. Eaton (1933) found a similar crest in a specimen of *Ambystoma macrodactylum,* as did de Villiers (1936); and Theron (1952) mentions it for *Ambystoma maculatum,* where it is much more fully developed. It is absent in *Rhyacotriton* (de Villiers, 1936).

The recessus acustico-facialis, housing the ganglion of the same name, is situated antero-ventrally in the otic capsule. The ganglion acustico-faciale is joined to the ganglion Gasseri by a ramus communicans. As in *Ambystoma maculatum* (Theron, 1952), the ramus palatinus of the facial nerve, on passing from the ganglion, proceeds forward through a foramen palatinum in the anterior floor of the otic capsule, whereas the ramus hyomandibularis extends backwards along the facialis canal and finally enters the cranioquadrate passage. The base of the facialis canal is formed by the cartilaginous processus basitrabecularis (Fig. 5). According to de Beer (1937) the medial wall of the facialis canal is formed by the prefacial commissure, and the lateral wall by the medial wall of the anterior cupola of the otic capsule. The post-palatine commissure separates the ramus hyomandibularis from the ramus palatinus. Although separated from the otic capsule by the capsular wall, the nerve seems to emerge from the otic cavity, a condition described by de Beer (1937) as primitive. Similar conditions prevail in *Salamandra* (de Beer, 1937) and *Ambystoma maculatum* (Theron, 1952). In *Triturus* de Beer (1937) describes the same "apparent" effect, the facial nerve also seeming to traverse the otic cavity, all the more so as the medial wall of the facial canal does not chondrify.

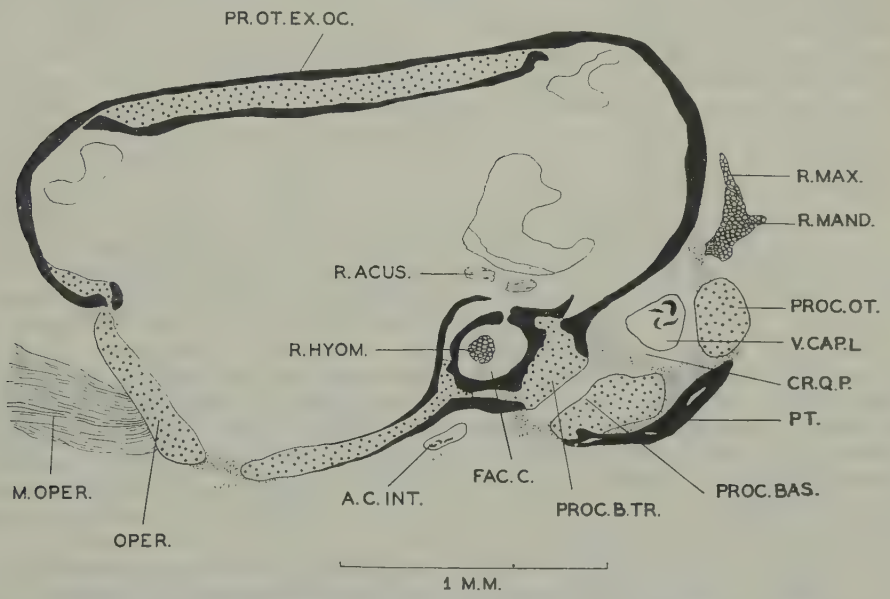

FIG. 5. Sagittal section through the otic capsule.
A.C.INT. arteria carotis interna; CR.Q.P. cranioquadrate passage; FAC.C. facialis canal; M.OPER. musculus operc ularis; OPER. operculum; PROC.BAS. processus basalis; PROC.B.TR. processus basitrabecularis; PROC.OT. processus oticus; PR.OT.EX.OC. prootic-exoccipital complex; PT. pterygoid; R.ACUS ramus acusticus; R.HYOM. ramus hyomandibularis VII; R.MAND. ramus mandibularis V; R.MAX. ramus maxillaris V; V.CAP.L. vena capitis lateralis.

The number of foramina through which the rami of the nervus acusticus enter the otic capsule, is subject to variation, both within the species and in the specimen itself. In the 50.5 mm. specimen examined, two foramina occur on the

right side and three on the left, though the extra, medially situated foramen is very small and is separated from the posterior one only by a narrow bony strip. Dorsally to the latter the completely ossified medial wall of the otic capsule is pierced by the ductus endolymphaticus, and posterior to this, at about the same height as the posterior foramen acusticum, by the ductus perilymphaticus.

Immediately lateral to the condylus occipitalis and situated in the exoccipital, is the foramen postoticum for transmission of the glossopharyngeal and vagus nerves (Fig. 2).

The fenestra ovalis is situated in the postero-ventral face of the auditory capsule (Fig. 2 & 5).

THE COLUMELLA AURIS

The sound-transmitting apparatus is typically ambystomid, without any notable divergences. It is composed of two completely independent elements: the columella and the operculum. The former consists of a medial, flattened foot-plate and a laterally projecting stylus columellae. The foot-plate, which is confluent with the antero-lateral wall of the fenestra ovalis, is hardly distinguishable from the otic capsule. Both stylus and foot-plate show perichondral ossifications confirming the findings of de Villiers (1938b) for the same species. A similar fusion to the otic capsule, and partial ossification, occur in *Onychodactylus* (Ryke, 1950), *Rhyacotriton* (de Villiers, 1938a) and *Ambystoma maculatum* (Theron, 1952). *Onychodactylus,* however, lacks an operculum (Ryke, 1950). In *Hynobius* and in *Cryptobranchus* (Dunn, 1922) the columella is free from the otic capsule. The lateral, distal portion of the stylus lies just ventral to the lateral face of the squamosal and close to the posterior surface of the pars quadrata palatoquadrati, to which it is connected by a ligamentum suspensorio-columellare. This ligament is replaced by a synchrondrosis in *Onychodactylus* (Ryke, 1950), *Rhyacotriton* (de Villiers, 1938a) and *Salamandra* (de Beer, 1937). In *Megalobatrachus* (Aoyama, 1930) the larval synchondrotic connexion between the stylus and pars quadrata completely disappears in the adult. According to recent opinions summarized by Reinbach (1950), the columella originates partly from the pars quadrata and partly from the otic capsule. Should this be true, as the evidence seems to indicate, the opinion held by both Kingsbury and Reed (1908) and Okutomi (1936), that the connexion between the columella and the pars quadrata is secondary, must be completely rejected; the same applies to the so-called primary connexion between the columella and the squamosal. The latter connexion is very weakly developed in *Ambystoma macrodactylum.*

As in most urodeles, with the exception of the *Perennibranchiata* (de Beer, 1937) the ramus hyomandibularis VII runs ventrally and in front of the well-developed ligamentum suspensorio-columellare. The stylus columellae separates the main trunk of the vena capitis lateralis, which runs on the dorsal side of the stylus, from its ventral rootlets scattered amongst the branches of the ramus hyomandibularis (Fig. 6). Taking these relations into account I cannot but agree with Theron (1952) in concluding that the so-called cartilaginous bar of an *Ambystoma* species, as described by de Villiers (1936), is the stylus columellae. Confirming this is the circumstance that this cartilaginous bar is connected by means of a ligament to the squamosal and pterygoid. Describing the sound-conducting apparatus de Villiers (1936, p. 235) says it is a "mere

cartilaginous operculum without any (plectral?) stalk, and is widely separate from the pars quadrata". It appears that he erroneously regarded the foot-plate of the columella as the operculum.

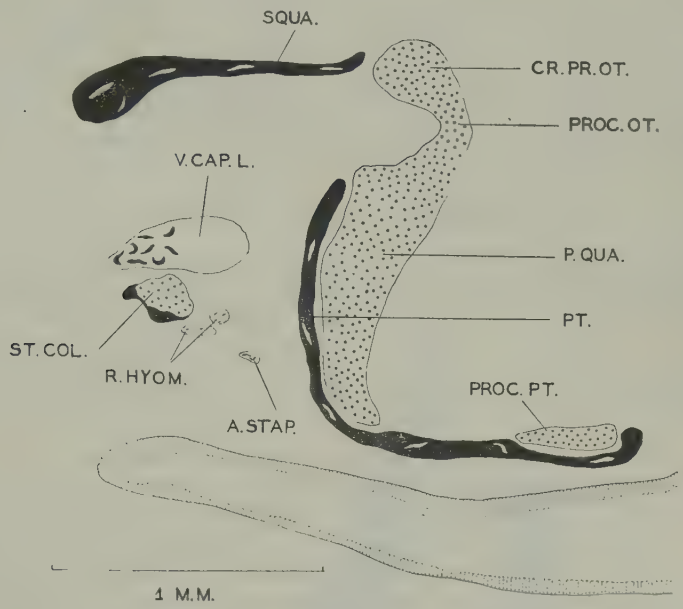

FIG. 6. Sagittal section through the suspensorial region. A.STAP. arteria stapedialis; CR.PR.OT. crista parotica; P.QUA. pars quadrata; PROC.OT. processus oticus; PROC.PT. processus pterygoideus; PT. pterygoid; R.HYON. ramus hyomandibularis VII; SQUA. squamosal; ST.COL. stylus columellae; V.CAP.L. vena capitis lateralis.

A very interesting feature concerning the relation of the vena capitis lateralis was found in the 63.5 mm. specimen. Normally the vein passes dorsally and then laterally to the stylus; in this particular case, however, the vein, after passing dorsally to the stylus, becomes incorporated between the stylus and the foot-plate, upon fusion of the former with the latter. It passes through the columella for only a very short distance and leaves it again in a lateral direction.

The operculum, which de Villiers (1938b) fails to mention in his paper on *Ambystoma macrodactylum,* lies free from any skeletal structures in the fenestra ovalis, except for the slight synchondrotic connexion on the dorsal fenestral edge. It is furthermore supported by a dense membrane, which surrounds it completely and fills the whole fenestra ovalis (Fig. 5). A similar connexion between operculum and otic capsule also occurs in *Ambystoma maculatum* (Theron, 1952), whereas the operculum of *Salamandra* (Francis, 1934), *Hynobius* and *Triturus* (Dunn, 1922) is quite free. Dunn (1922) states that the operculum is ossified in adult *Ambystoma,* but in the species examined by Theron and myself the operculum remains cartilaginous. An operculum is absent in *Rhyacotriton* (Dunn, 1941), *Cryptobranchus* (Kingsbury and Reed, 1909) and *Onychodactylus* (Ryke, 1950). There is some uncertainty about the origin of the

operculum, but Kingsbury and Reed (1909) and Reinbach (1950) regard it as coming from the otic capsule. In the *Plethodontidae* and *Desmognathidae* the columella and the operculum are fused (Stadtmüller, 1936).

Attached to the posterior face of the operculum, is the musculus opercularis (Fig. 5), stretching backwards as far as the suprascapula, to which it is attached. In forms such as *Rhyacotriton,* where an operculum is absent, there is no muscular connexion between the columella auris and the shoulder-girdle (Dunn 1941). The musculus opercularis appears to be absent in all urodeles in the larval state, and is only developed as an adaptation to terrestrial life (Kingsbury and Reed, 1909).

THE SUSPENSORIAL REGION

The palatoquadrate consists of a slender forwardly projecting pars palatina, represented by the processus pterygoideus, and a comparatively larger pars quadrata. The processus pterygoideus which lies in a groove on the dorsal face of the pterygoid, has already been mentioned under the membrane bones of the olfactory region. It does not overlap the posterior end of the maxillary as in *Ambystoma maculatum* (Theron, 1952), but ends a short distance behind this bone.

The pars quadrata is connected to the neurocranium by the processus ascendens, the processus basalis and the processus oticus. The ramus hyomandibularis VII, the vena capitis lateralis and the arteria stapedialis normally pass through the cranioquadrate passage (Fig. 5); in *Ambystoma maculatum* (Theron, 1952) and in *Ambystoma macrodactylum,* however, the arteria stapedialis runs ventral to this passage. As in *Rhyacotriton* (de Villiers, 1938), *Onychodactylus* (Ryke, 1950), *Salamandra* (Francis, 1934), and *Ambystoma maculatum* (Theron, 1952), the processus ascendens is synchondrotically connected to the pila antotica (Fig. 1). The process has the usual relationships to the branches of the trigeminal and to other structures in its vicinity. Stadtmüller (1936) mentions that perichondral ossifications occur in the processus ascendens of *Salamandra,* an observation confirmed by Francis (1934).

On the dorso-lateral face of the otic capsule the cartilaginous otic process is synchodrotically fused with the crista parotica (Fig. 6), as described by de Villiers (1938b). It is situated laterally to the processus ascendens and directly opposite it. The processus oticus which is confluent with the crista parotica lies dorso-laterally to the vena capitis lateralis and antero-laterally to the ramus hyomandibularis VII. Similar conditions occur in *Ambystoma maculatum* (Theron, 1952), in *Onychodactylus* (Okutomi, 1936; Ryke, 1950) and in certain species of *Ambystoma* (de Beer, 1937). According to Wintrebert (1922) in the metamorphosed *Ambystoma* the otic connexion becomes discontinuous (cp. Aoyama (1930) for *Megalobatrachus).* In *Rhyacotriton* (de Villiers, 1938a) only traces of a vestigial processus oticus can be discerned.

Forming the ventral boundary of the cranioquadrate passage, and consequently lying ventrally to the vena capitis lateralis and the ramus hyomandibularis, is the processus basalis (Fig. 5). It has a diarthrosis with the basitrabecular process, which, according to Gaupp, is a primitive condition (Stadtmüller, 1936). The joint cavity between the processus basalis and the processus basitrabecularis, as seen in transverse section, is somewhat S-shaped; furthermore, the articular surfaces of both processes are not even, thus practically

eliminating any rotational movement. Similar diarthrotic connexions, occur in *Ambystoma maculatum* (Theron, 1952), *Chioglossa,* and *Hynobius* (Stadtmüller, 1936). In *Onychodactylus* Stadtmüller (1936) found a diarthrosis on one side and a fusion on the other, whereas Ryke (1950) described differently developed diarthroses on both sides. A confluent basal connexion is found in *Triturus, Salamandra maculosa* (de Beer, 1937). *Diemictylus, Plethodon,* and *Desmognathus* (Stadtmüller, 1936).

The posterior edge of the pars quadrata is also connected by means of a strong ligament to the ceratohyal, an epihyal being absent. The latter element seems to be subject to variation. Eaton (1933) found it in *Ambystoma macrodactylum, Ambystoma gracile,* and *Rhyacotriton.* In the specimen of *Ambystoma macrodactylum* which de Villiers (1938b) examined, the epihyal was present only on one side, and the specimen of *Rhyacotriton* examined by him (1938a) had no such element at all. The ligamentum suspensorio-columella, connecting the pars quadrata and the sound-conducting apparatus, has been mentioned.

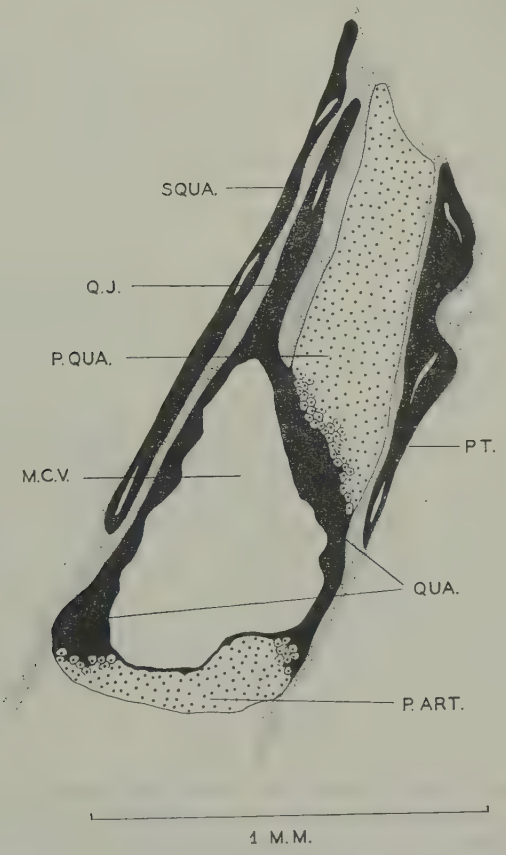

FIG. 7. Transverse section showing invasion of the quadrate region by the quadratojugal. M.C.V. marrow cavity; P.ART. pars articularis; P.QUA. pars quadrata; PT. pterygoid; QUA. quadrate; Q.J. quadratojugal; SQUA. squamosal.

The quadrate, the only ossification of the palatoquadratum, is situated dorsally to the pars articularis and has a large marrow cavity (Fig. 7). The quadrate is not very large, and dorso-laterally the ventral portion of the quadratojugal is indistinguishably fused with it (Fig. 7). According to Eaton (1933) a separate quadrate ossification is absent in *Ambystoma gracile,* apparently having become fused with the squamosal.

There are three membrane bones of the palatoquadrate: the pterygoid, the quadratojugal, and the squamosal. The pterygoid, whose shape and relations are shown in figures 1 and 2, stretches from the posterior end of the pars quadrata to about the middle of the processus ptergoideus. Its palatal ramus underlies the processus pterygoideus; its quadrate ramus extends onto the pars quadrata and vertically upwards along its posterior face (Fig. 6). This vertical portion incompletely separates the pars quadrata from the processus basalis, but the small horizontal part of the ramus does not completely cover the processus basalis as it does in *Ambystoma maculatum* (Theron, 1952). Anteriorly the pterygoid is widely separated from the maxillary. The quadratojugal and the squamosal tend to cover the suspensorial region dorso-laterally.

The quadratojugal consists of two regions: an upper, which is separated from the squamosal and the pars quadrata by means of connective tissue (Fig. 8), and a lower, which has invaded the quadrate to such an extent that a continuous skeletal entity is formed (Fig. 7). The lower region of the quadratojugal is therefore a mixed bone. Similar conditions are found in *Ambystoma maculatum* (Theron, 1952) and in *Onychodactylus* (Ryke, 1950), though in the latter and in *Hynobius* (de Villiers, 1936) synostosis takes place between the quadrajugal and the squamosal; de Villiers (1938b) describes a similar synotosis in *Ambystoma macrodactylum.* In the 50.5 mm. specimen examined, only extremely weak signs of synostosis can be discerned. In the other specimen this part is unfortunately damaged. A processus zygomaticus of the squamosal is absent in urodeles (de Villiers, 1936), though Theron (1952) describes one in *Ambystoma maculatum.* At the extreme median edge of the squamosal there is a very slight synostosis with the prootic on the right side of the 50.5 mm. specimen. This together with the squamosal-quadratojugal connexion completely eliminates the so-called pivoting action of the squamosal as described by Eaton (1933), and makes any movement of the squamosal improbable.

Turning now to movement of the palatoquadrate, one finds that this too is well-nigh impossible. The upper portion of the palatoquadrate is so firmly fused with the neurocranium by means of the processes ascendens and the processus oticus, that the movement allowed by the processus basalis, and perhaps the ligamentum suspensorio-columellare, is so slight that, strictly speaking, *Ambystoma macrodactylum* is monimostylic, a conclusion also drawn by de Villiers (1938b) for the same species, and for *Rhyacotriton* (1938a).

LOWER JAW

The lower jaw is composed of two membrane bones, the dentary and the gonial, which form a canal, the canalis primordialis, containing Meckel's cartilage, the ramus mandibularis V, the ramus alveolaris VII, and bloodvessels.

Meckel's cartilage is a rod-like structure which stretches throughout the length of the lower jaw, and fuses at the mental symphysis with its partner from the other side. It is almost completely enveloped anteriorly by the dentary

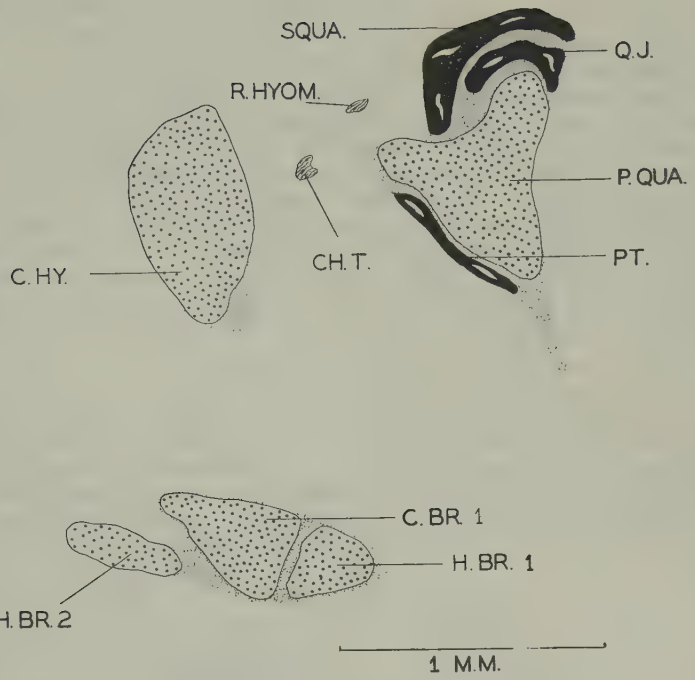

FIG. 8. Sagittal section through the suspensorial region (more lateral than Fig. 6).
C.BR.1 ceratobranchial 1; CH.T. chorda tympani; C.HY. ceratohyal; H.BR.1 hyobranchial 1; H.BR.2 hyobranchial 2; P.QUA pars quadrata; PT. pterygoid; Q.J. quadratojugal; R.HYOM. ramus hyomandibularis VII; SQUA. squamosal.

and posteriorly by the gonial, leaving exposed only the pars articularis and a portion anterior to the articular. The distal end of the cartilage ossifies as a cartilage bone, the mentomandibular, which, as in *Megalobatrachus* (Aoyama, 1930), *Ambystoma maculatum* (Theron, 1952), and *Onychodactylus* (Stadtmüller, 1936), is indistinguishably fused with the dentary, thus forming a mixed bone. In *Ambystoma tigrinum* (Gaupp, 1911) the mentomandibular remains independent. A marrow cavity occurs in the mentomandibular-dentary complex.

The articular is not free, as it is in *Salamandra* (Gaupp, 1911) and *Ambystoma tigrinum* (Stadtmüller, 1936), but is fused with the gonial to form a mixed bone, the gonioarticular. This also occurs in *Ambystoma maculatum* (Theron, 1952), *Megalobatrachus* (Aoyama, 1930) and *Triturus* (Gaupp, 1911). The extreme proximal end of Meckel's cartilage, however, remains cartilaginous and articulates with the pars quadrata. *Necturus maculatus* (Stadtmüller, 1936) is completely devoid of any particular ossification. Apart from the connexion with the gonial, the articular may even be fused with the dentary, forming a dentogonioarticulare as in *Ambystoma opacum* (Stadtmüller, 1936).

The dentary, largest of the two investing bones, covers most of Meckel's cartilage laterally, ventrally, and dorsally. It stretches from the median symphysis, where it is synostotically connected to the mentomandibular, tapers

backwards, and terminates postero-laterally to the articular. It is the only tooth-bearing bone of the lower jaw, and medially it almost completely surrounds Meckel's cartilage in the canalis primordialis. Anteriorly the dentary is perforated by a number of foramina dentofacialia, for transmission of blood-vessels and branches of the ramus mandibularis V.

The other investing bone, the gonial, lies medially to the dentary and Meckel's cartilage, thus also helping to form the canalis primordialis. Posteriorly it stretches back as far as the articular, to which it is fused, and broadens out medially to form the processus coronoideus, to which muscle fibres are attached.

The ramus alveolaris VII (chorda tympani) passes through the canalis chordae tympani in the ventral part of the gonial and enters the canalis primordialis. The ramus mandibularis V does not enter the canalis primordialis through a canal or foramen, but simply passes in between the dentary and Meckel's cartilage. In *Siren* (Hentschel, 1936) the chorda tympani remains outside the canalis primordialis.

HYOBRANCHIAL APPARATUS

There seems to be some variation in the hyobranchial apparatus of the *Urodela,* but except for minor divergencies, which will be referred to in the course of this discussion, the hyobranchial apparatus of *Ambystoma macrodactylum* largely agrees with that of *Ambystoma maculatum* (Theron, 1952).

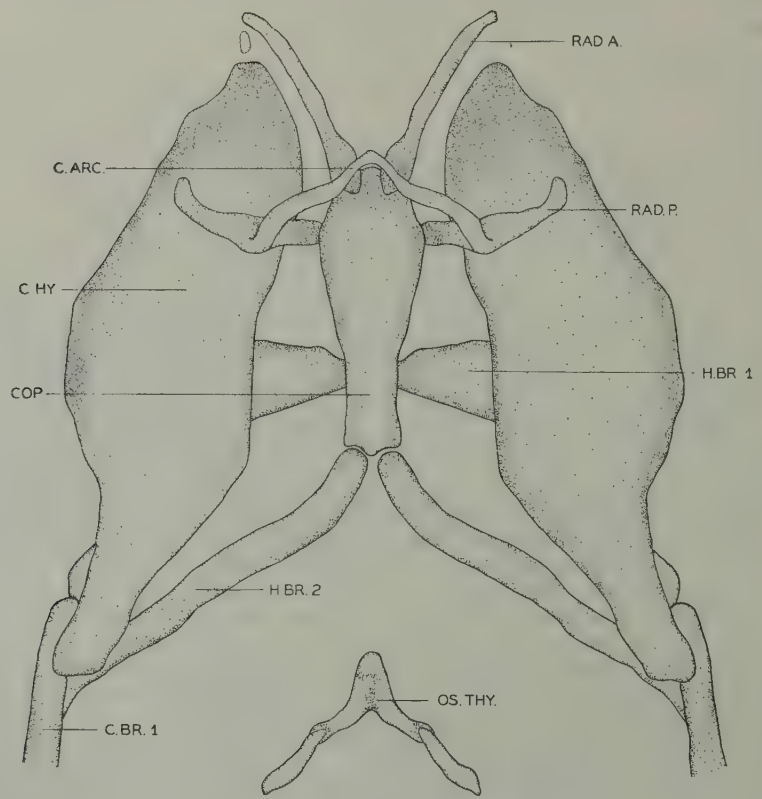

FIG. 9. Dorsal view of the hyobranchial apparatus. x approx 14.8 C.ARC. cartilago arcuata; C.BR.1 ceratobranchial 1; C.HY. ceratohyal; COP. copula; H.BR.1 hyobranchial 1; H.BR.2 hyobranchial 2; OS.THY. os thyreoideum; RAD.A. radius anterior; RAD.P. radius posterior.

The hyoid arch consists of two elements, a small hypohyal and a much larger ceratohyal (Fig. 9). The former, a rod-like radius anterior, is the foremost of the two structures and is in a weak synchondrotic connexion with the copula, from which it stretches in an antero-dorso-lateral direction terminating opposite the anterior tip of the broad, flat ceratohyal, with which it is connected by means of a ligament. This condition Gaupp (1904) describes as primitive. In *Ambystoma maculatum* (Theron, 1952) the connexion between the radius anterior and the copula is syndesmotic. An intervening piece of cartilage occurs in the ligament connecting the radius anterior and the ceratohyal, though curiously enough only on the left side of the 50.5 mm. specimen. In *Salamandra* (Gaupp, 1904) there is no ligament between the two structures, in *Onychodactylus* (Fukuda, 1930) the hypohyal and ceratohyal are fused, whereas in *Triturus* and *Spelerpes* (Gaupp, 1904) a hypohyal is absent. As already mentioned, the ceratohyal is connected to the palatoquadrate by means of a strong ligamentum hyoquadratum. There is apparently an intervening epihyal, such as described by both Eaton (1933) and de Villiers (1938) for this species.

The copula or basibranchiale of Wiedersheim (1877) is the median cartilage with which the visceral arches articulate (Fig. 9). Posterior to the radius anterior, and synchondrotically connected to the lateral side of the copula, is the radius posterior, a horn-like, laterally-protruding cartilage, which lies dorsally to the ceratohyal and completely free from it. The radii posteriores which arise secondarily during metamorphosis, according to Drüner (Francis, 1934), are dorsally connected by a V-shaped cartilago arcuata, which is absent in *Salamandra* (Gaupp, 1904). Radii posteriores are entirely lacking in the Asiatic salamandrids (Gaupp, 1904).

Of the four branchial arches present in the larval *Ambystoma* (Gaupp, 1904) only two remain in the adult: the first, consisting of both hypobranchial and ceratobranchial, the second of a hypobranchial only (Fig. 8). Again the Asiatic salamandrids form an exception; they have both a hypobranchial II and a ceratobranchial II (Gaupp, 1904), a condition described by Wiedersheim (1877, p. 497) as a „niedrige Organisationsstufe". The connexion between the branchial arches and the copula, is syndesmotic, differing from the synchondrotic fusion in *Ambystoma maculatum* (Theron, 1952). In *Salamandra* (Francis, 1934) the hypobranchials and the ceratobranchial of the first branchial arch are homocontinuously fused, and the hypobranchial II is again fused to the previous arch.

The "Copulastiel" of the copula disappears during metamorphosis, leaving only the forked posterior portion, which ossifies as the os thyreoideum (Gaupp, 1904), but its posterior epiphyses remain cartilaginous (Fig. 9). In *Ambystoma maculatum* (Theron, 1952) the os thyreoideum lies just posterior to the copula and ventrally to the hypobranchial II; in *Ambystoma macrodactylum* and in *Salamandra* (Gaupp, 1904) it is situated far more posteriorly.

SUMMARY

1 A septum nasale is absent.
2 A processus praenasalis inferior lateralis is present; a median unpaired processus praenasalis superior medius is lacking.
3 A broad planum internasale takes the place of the fenestra praecerebralis, which is absent.
4 The fenestra lateralis nasi does not communicate with the incisura ectochoanalis.

5 The crista rostrocaudalis is fused with the solum nasi, forming a dorso-lateral support to "Jacobson's organ".
6 The ductus nasolacrimalis runs along the impressio conchalis and passes through both the prefrontal (lacrimal?) and the septomaxillary.
7 The vomerine teeth are arranged in medial and lateral groups on the posterior border of the vomer.
8 A palatine is absent.
9 The nervus abducens does not have its own foramen but accompanies the trigeminal through the foramen prooticum.
10 The sclerotic cartilage is well developed.
11 The supratemporal crest is small.
12 Two fenestrae basicraniales posteriores are present.
13 The exoccipital and the prootic are fused to form an exoccipito-prootic complex.
14 Both the n. glossopharyngeus and the n. vagus pass through the foramen postoticum.
15 The columella auris consists of a columella and an operculum.
16 The foot-plate of the columella is fused with the otic capsule, and the stylus is attached to the pars quadrata by the ligamentum suspensorio-columellare.
17 In one specimen the vena capitis lateralis is enclosed between the stylus and the foot-plate of the columella.
18 The operculum has a slight synchondrotic connexion with the dorsal edge of the fenestra ovalis.
19 A musculus opercularis which connects the operculum and the shoulder girdle, is present.
20 The processus ascendens is synchrondrotically attached to the pila antotica.
21 The processus oticus is fused with the crista parotica.
22 The processus basalis has a diarthrotic connexion with the basitrabecular process.
23 An epihyal is absent.
24 The quadrate and the quadratojugal are fused.
25 There are weak signs of synostosis between the quadratojugal and the squamosal.
26 A processus zygomaticus of the squamosal is absent.
27 The dentary and the gonial are the only membrane bones of the lower jaw.
28 The cartilage bones, mentomandibular and articular, are fused with the dentary and the gonial respectively.
29 The radii posteriores of the hyobranchial skeleton are joined together by a V-shaped cartilago arcuata.

ACKNOWLEDGEMENTS

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EXPLANATION OF LETTERING

A. C. INT.	arteria carotis interna
A. STAP.	arteria stapedialis
C. A. OR.	cartilago antorbitalis
C. ARC.	cartilago arcuata
CAV. IN. N.	cavum internasale
C. BR. I.	ceratobranchial I
C. CUP.	cartilago cupolaris
C. EC. CH.	cartilago ectochoanalis
CH. CAR.	chorda cartilage
CH. T.	chorda tympani
CH. TIS.	chorda tissue
C. HY.	ceratohyal
C. INF. NAR.	cartilago infranarina
C. OBL.	cartilago obliqua
COL. ETH.	columella ethmoidalis
CON. TIS.	connective tissue
COP.	copula
CR. PR. OT.	crista parotica
CR. Q. P.	cranioquadrate passage
CR. R. C.	crista rostrocaudalis
CR. SEL.	crista sellaris
C. RT. NAR.	cartilago retronarina
D. N. L.	ductus nasolacrimalis
FAC. C.	facialis canal
F. D. N. L.	foramen for ductus nasolacrimalis
FEN. BC. A.	fenestra basicranialis anterior
FEN. BC. P.	fenestra basicranialis posterior
FEN. B. NAS.	fenestra basalis nasi
FEN. D. NAS.	fenestra dorsalis nasi
FEN. L. NAS.	fenestra lateralis nasi
FEN. NAR.	fenestra narina
F. OC.	foramen oculomotorium
F. O. N. L.	foramina orbitonasalia lateralia
F. O. N. M.	foramen orbitonasale mediale
F. OP.	foramen opticum
F. PAL.	foramen palatinum
F. P. OT.	foramen postoticum
F. PR. OT.	foramen prooticum
FR.	frontal
F. R. L. NAS.	foramen for ramus lateralis nasi V (a)
F. R. M. NAS.	foramen for ramus medialis nasi V (a)
H. BR. 1	hypobranchial 1
H. BR. 2	hypobranchial 2
HYP. COM.	hypochordal commissure
INS. EC. CH.	incisura ectochoanalis
LACR.	lacrimal
MAX.	maxillary
M. C. V.	marrow cavity
M. OPER	musculus opercularis

NAS.	nasal
OPER.	operculum
ORB. SPH.	orbitosphenoid
OS. THY.	os thyreoideum
PAR.	parietal
P. ART.	pars articularis
PL. CON.	planum conchale
P. QUA.	pars quadrata
PREM.	premaxillary
PR. FR.	prefrontal
PROC. ASC.	processus ascendens
PROC. BAS.	processus basalis
PROC. B. TR.	processus basitrabecularis
PROC. OT.	processus oticus
PROC. P. NAS. INF. LAT.	processus praenasalis inferior lateralis
PROC. PT.	processus pterygoideus
PR. OT. EX. OC.	prootic-exoccipital complex
P. SPH.	parasphenoid
PT.	pterygoid
Q. J.	quadratojugal
QUA.	quadrate
R. ACUS.	ramus acusticus
RAD. A.	radius anterior
RAD. P.	radius posterior
R. HYOM.	ramus hyomandibularis VII
R. LAT.	ramus lateralis nasi V (a)
R. MAND.	ramus mandibularis V
R. MAX.	ramus maxillaris V
SEY. PAL. PROC.	Seydel's palatal process
S. MAX.	septomaxillary
SQUA.	squamosal
ST. COL.	stylus columellae
TEC. SYN.	tectum synoticum
T. IN. N.	tectum internasale
TUB. INT.	tuberculum interglenoidale
V. CAP. L.	vena capitis lateralis
VOM.	vomer

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THE DEVELOPMENT OF THE CHONDROCRANIUM OF THE OSTRICH

by

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with 30 Text-figures.

Submitted: November 1953.

Abstract

The chondrocranium of the ostrich is typically avian. There is no posterior basicranial fenestra in the perichordal plate, the latter being continuous with the cochlear portions of the otic capsules. The acrochordal cartilage develops after the appearance of the perichordal plate; the former is continuous laterally with the pilae antoticae. The pilae antoticae spuriae are weakly developed. The hypocentra of at least two absorbed occipital vertebrae are present posteriorly in the perichordal plate. The hypocentra of only the axis and atlas develop beyond a precartilaginous condition. The vagus and glossopharyngeal nerves emerge fundamentally through a common foramen. The basitrabecular processes arise as independent anlagen. No separate polar cartilage anlagen are observed. The interorbital septum is continuous. A supraorbital cartilage connects the two regions of the orbital cartilage during the greater part of the ontogeny. The olfactory nerves come to lie within the orbit after the degeneration of the orbital cartilages anteriorly. These nerves are covered by the parietotectal cartilage which grows back on the dorsal edge of the interorbital septum. A well-developed processus maxillaris posterior of the nasal capsule is present and later ossifies as the os uncinatum. The hyoid arch arises as an unsegmented mesenchymatous rod.

CONTENTS

I. INTRODUCTION

This work was undertaken at the suggestion of Prof. C. G. S. de Villiers with a view to complementing the work already done by Crompton (1953) on the penguin chondrocranium, and to confirm the results of an earlier investigation carried out by Brock (1937). The latter showed that, as far as could be ascertained from the limited material at her disposal, the ostrich chondrocranium differed but very little from that of carinate birds. The ultimate object of all these investigations was to cast some light on the problematical phylogeny of the *"Ratitae"* or *Dromaeognathae*. The limited literature on the subject was recently and very adequately reviewed by both de Beer and Barrington (1934) and Crompton (1953). In the wider sphere the literature dealing with the problems of phylogeny has been discussed by Lowe (1928, 1933 and 1942) and de Villiers (1946). For further information on the earlier literature the reader is referred to these works.

In order to give a more coherent account of the development, the chondrocranium in the individual regions has been described rather than in arbitrary stages which are really "an abstraction of the four dimensional space-time phenomenon which a living organism is" (de Beer, 1940, p. 7). Discussion of and comparison with conditions in other vertebrate groups is reserved for the end of each section. Finally, a discussion at the end of the work deals with avian phylogeny in the light of the evidence derived from craniogenesis.

Only a limited number of reconstructions of the chondrocranium is given, since this has been amply illustrated in previous papers (See de Beer and Barrington, 1934, Crompton, 1953 et al.). Structures that have an involved topography as well as those which differ markedly in other birds are, however, figured in detail, both sectional and reconstructional drawings being used.

II. MATERIAL AND TECHNIQUE

The material used in this investigation consisted of some 23 ostrich embryos collected in the Oudtshoorn district of the Cape Province. The parent birds, bred in captivity, may possibly represent a cross with the North African ostrich, as a flock was brought out at the beginning of the century to improve the local stock. Except for a few of the older embryos collected from nests, the eggs were all incubated artificially. Farmers think it essential to keep the eggs at least 48-72 hours, turning them every 12, after collecting them in the field and before placing them in the incubator. The eggs are incubated at 103°F., the air in the incubator being kept as humid as possible. They were turned every 12 hours, when they were also ventilated for about 20 minutes.

All specimens were fixed and preserved in Allen's fluid, P.F.A.$_3$ (McClung, 1937). This has the advantage of being an excellent fixative, and at the same time material can be preserved in it over long periods without danger of overfixation. In addition, the presence of picric and acetic acid in

the fixative renders it unnecessary to decalcify the embryos. The removal of the fixative may be facilitated by adding a trace of lithium carbonate to the alcohol.

Since it was probable that the eggs had been partially incubated before collection the exact age of the embryos was uncertain; they were consequently classified according to cranial length. Serial sections were made of embryos of the following cranial length:—

Sectioned sagittally: 3.3 mm., 4.0 mm., 5.4 mm., 6.8 mm., 7.8 mm., 8.4 mm., 8.7 mm., 9.2 mm., 10.7 mm., 11.0 mm., 11.9 mm., 12.4 mm., 15.3 mm., 21.0 mm.

Sectioned transversely: 12.3 mm., 15.5 mm., 17.3 mm., 19.0 mm., 20 days, 21 days and 34 days old.

Sectioned frontally: 8.2 mm., 11.6 mm.

Almost all the embryos were very lightly stained in toto with Mayer's acid haemalum and the sections counterstained with Bismark-brown and eosin. This proved to be a simple and completely efficacious stain combination, giving uniformly good results with excellent differentiation. In order to obtain maximum colour contrast and differentiation with sections stained with a blue-yellow combination of this sort, it was found to be essential to examine them with a blue filter in the microscope, the intensity of the blue required in the lighting being dependent on the intensity of the haemalum staining. The Nowikoff method advocated by Crompton was tried, but without success. A few of the older embryos in which dermal bones were present, were bulk-stained with borax carmine and counterstained with azan.

The occasional use of a phase contrast microscope failed to disclose any structures which had not already been successfully differentiated by the staining methods employed.

The graphic and contour reconstructions were made according to the system described by Pusey (1939), which is used extensively in this Institute. Wax-plate reconstruction models were made of the nasal capsule of the ostrich and of the South African night-jar.

For the sake of comparison the nasal capsules of an adult *Rhea americana* kindly supplied by Prof. H. Steiner of the Cantonal University, Zürich, was microtomised at 50 μ. In addition a young embryo and the nasal capsules of a nestling of the South Africa night-jar, *(Nyctisyrigmus pectoralis pectoralis)* sectioned by Dr. M. E. Malan of this Institute were available for purposes of comparison. The embryo corresponds roughly to the stage of development attained to by an ostrich embryo of 21 mm. The developmental series made by F. J. Grewe of the duck *(Anas)*, A. W. Crompton of the penguin *(Spheniscus demersus)*, and D. van Z. Engelbrecht of the Red Bishop bird *(Pyromelana orix)* were also available for study.

III. ACKNOWLEDGEMENTS

I would like to express my sincere thanks to Prof. C. G. S. de Villiers for his constant advice and constructive criticism and to Prof. C. A. du Toit for his personal interest and assistance in the preparation of this manuscript.

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Finally I am indebted to the Council for Scientific and Industrial Research for a research scholarship, without which the expenses entailed in this investigation could not have been met.

IV CHONDROCRANIAL ONTOGENESIS

(a) *THE PERICHORDAL PLATE, ACROCHORDAL AND METOTIC CARTILAGES*

(i) *Description.*

Owing to insufficient material it was not possible to study the early anlagen of the occipital and basal regions of the skull in any great detail. For the same reason the problem of cranial segmentation is only touched upon. The early development of the anterior cervical and occipital vertebrae in relation to the occipito-atlantic joint is briefly dealt with.

In the earliest stage studied, that of a 3.3 mm. embryo, approximately 5 days old, no cartilage has been laid down. The sclerotomes, however, separated from each other by the intersclerotomic fissure, are very clear, lying on either side of the chorda as mesenchymatous thickenings. A thin homogenous chordal sheath slightly thickened immediately below the spinal cord is present. The chorda exhibits slight lateral intrasclerotomic constrictions in the cervical region. The intermetameric bloodvessels may be seen passing between the sclerotomes. Situated dorsally above each sclerotome there is an epithelioid dermatomyotome, which is already changing into muscle fibres laterally. The sclerotomes themselves are distinctly divided into cranial and caudal sclerotomites by a narrow intrasclerotomic fissure. In the dorso-median part of each cranial sclerotomite may be seen the segmental anlagen of the spinal ganglia.

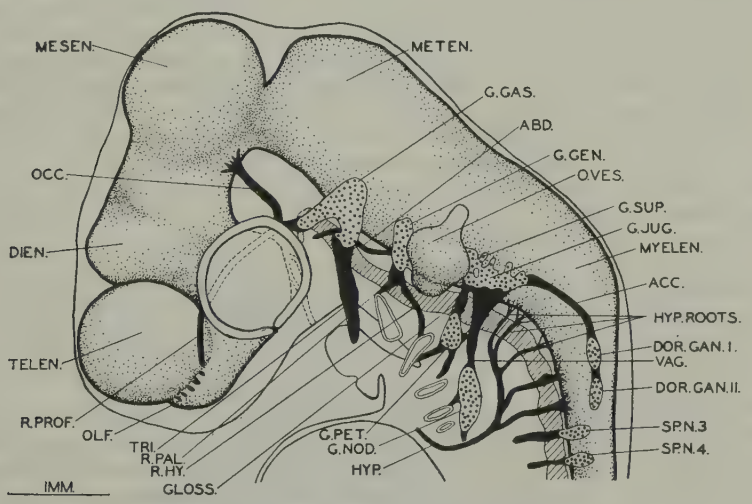


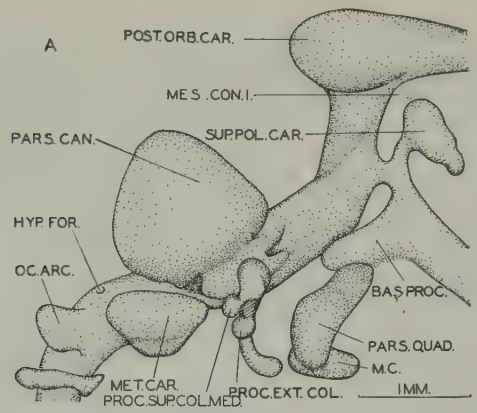
FIG. 1
Ostrich embryo 4.0 mm. lateral reconstruction of brain and cranial nerves (semi-diagrammatic).

By the time the 4 mm. stage is reached both the perichordal plate (see discussion) and the anlagen of the last two occipital vertebrae are present. At the point where the occipito-atlantic joint will eventually be formed there is a distinct flexure ("Nackenbeuge", Sonies, 1907) between the steeply rising cervical region and the occipital region. Between the otic vesicles the perichordal plate lies in a more or less horizontal plane rising again in its more anterior parts to form an almost vertical wall behind the hypophysis cerebri. There is only the faintest mesenchymatous condensation indicating the future position of the acrochordal cartilage. The perichordal plate extends only as far as the morphological anterior tip of the chorda. Anteriorly this plate does not bifurcate to form a basicranial fenestra as in many other forms. In the ostrich the chorda lies in the dorsal portion of the perichordal plate and is irregularly curved in the sagittal plane. In common with all vertebrates with extreme cranial flexure (Gaupp, 1906), the most anterior tip of the chorda curves ventrally, projecting from the morphological ventral surface of the perichordal plate. The segmental perichordal rings (Piiper, 1928) of only the last two absorbed cranial vertebrae can be made out. In this stage, however, these "rings" are incomplete both dorsally and ventrally while those of the atlas and succeeding vertebrae are only open dorsally. The chorda in the cervical region is now slightly constricted and has a monoliform appearance. The slight dilatations are surrounded anteriorly by the perichordal rings. The intrascleretomic fissures are less in evidence than in the previous stage and have become very narrow.

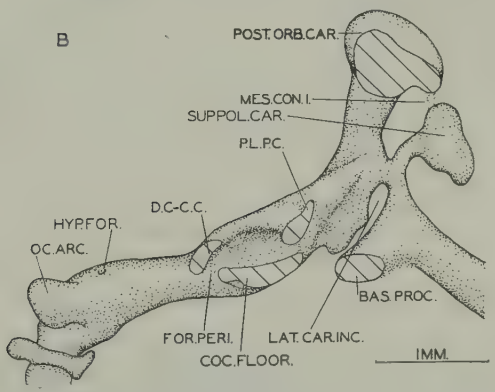
In this stage (4 mm.) the first mixed spinal nerve is the third cervical (Fig. 1). The segmental ganglia of the first two are united by a collector nerve from the vagus, the spinal accessory. Of this nerve Haller von Hallerstein (1934, p. 654) remarks: "Der N. accesorius der Vögel ist ein ganz dünner Nerv; seine Wurzelbündel sind vollständig mit dem des N. vagus vereinigt". This was also observed by Jager (1926). The fibres from these two spinal ganglia as well as those of the spinal accessory consequently emerge from the skull together with the vagus in much the same way as in mammalian embryos. Their corresponding ventral roots emerge in the usual way between the anlagen of the proatlas, atlas, and axis, unite with the three hypoglossal nerve roots, and pass round behind the visceral pouches to end immediately ventral to them. Even in the earliest stage studied (3 mm.) no more than 3 occipital nerve roots or their corresponding myomeres were observed. The condition as described above persists up to the 15 mm. stage when the second cervical ganglion, maintaining its connexion with the accessory nerve, also makes contact with its ventral root; in this way a mixed nerve apparently arises. In the ostrich, as in the gull *(Larus)*, "the single reliable criterion in distinguishing between the atlas (first) and axis (second) sclerotomes from other cervicals is the rudimentary condition or absence of their spinal ganglia" (Piiper, 1928, p. 296).

When the head of the embryo has reached a length of 5.4 mm. the acrochordal cartilage is reasonably well developed, and from it the pilae antoticae project in a lateral direction. Between their bases the dorsal edge of the acrochordal is pierced in a dorso-ventral direction by a pair of channels accommodating the oculomotor nerves. The basal plate is becoming more heavily chondrified throughout and is continuous laterally with the still largely mesenchymatous otic capsules. Anterior to the capsules the basal plate narrows

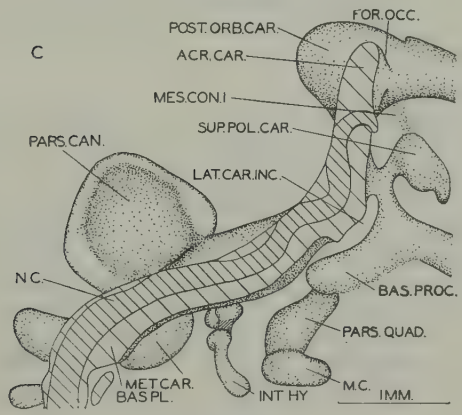
FIG. 2: Ostrich embryo 9.2 mm.



A. Lateral reconstruction of the basal plate. The anterior region of the chondrocranium has been removed.



B. Lateral reconstruction of the basal plate. The anterior region of the chondrocranium and otic capsule have been removed.



C. Median reconstruction of basal plate. The anterior region of the chondrocranium has been removed.

slightly, and allows the branches of the trigeminal and facial as well as the abducent nerve (which latter in this stage lies lateral to the base of the pila antotica) to pass through the still widely open prootic fissure. In the occipital region there are on either side of the basal plate three pairs of grooves which accommodate the hypoglossal roots; the vagus and glossopharyngeal nerves emerge through the open fissura metotica. The hypocentral elements of the last two absorbed occipital vertebrae, and especially those of the atlas and axis, are easily distinguishable (Fig. 3A). Anterior to the first absorbed occipital vertebra there is a slight concentration of cells in the cartilage of the basal plate. It is possible that this represents the hypocentrum of the absorbed occipital vertebra of the 7th segment (segmentation according to de Beer and Barrington, 1934). The proatlas still appears as an independent cartilaginous ring, open dorsally at the end of the occipital region, and with its basidorsal elements represented by small mesenchymatous stumps. The intrasclerotomic constrictions of the notochord are becoming more accentuated, especially ventrally and laterally. Three such constrictions are present in the posterior occipital region of the basal plate, the corresponding intersclerotomic dilatations lying between the 7th and 8th, and 8th and 9th absorbed occipital sclerotomes or segments. Owing to continued growth the perichordal rings lose their independence and form a continuous perichordal tube (Piiper, 1928) with segmental ventro-lateral expansions. This results in the obliteration of the intersclerotomic fissures, which are now marked by the intersclerotomic bloodvessels. The intrasclerotomic fissures, though narrow, are none the less visible in the more posterior cervical perichordal rings. They are, however, absent between the proatlas, atlas and axis anlagen. The caudal sclerotomites (hypocentral elements) are better developed than their cranial halves (pleurocentral elements). The more dorsal portions of the caudal sclerotomites (basidorsals) are still mesenchymatous and lie between successive spinal ganglia.

In the 6.8 mm. stage something quite unusual appears. In this particular specimen the hypoglossal roots on both sides of the head join together immediately below and lateral to the brain and pass anteriorly as a single pair of nerves mesial to the vagus nerves and postero-ventrally to the otic sacs to end there. They make no connexion with the first two cervical ventral nerve roots as in other embryos. The usual hypoglossal fissures are present in the basal plate but no nerves pass through them. As far as could be ascertained this embryo is normal in all other respects. In the anterior cervical region the intrasclerotomic fissures no longer exist, owing to further growth of the perichordal tube.

As growth proceeds the hypocentral elements of the proatlas, atlas, and axis continue to differentiate until in the 8.4 mm. stage; they are larger than those of the succeeding vertebrae. The hypocentra of only the atlas and axis develop beyond a precartilaginous condition. Soon after the 9.2 mm. stage recession occurs in the rest, and they disappear. The basidorsals, attached by dense precartilage to their respective hypocentra, now separate the segmental spinal ganglia from each other, but do not yet meet above the spinal cord. Above the ganglia they are attached by dense mesenchyme to those lying anterior and posterior to them. Similarly the last occipital arch has also grown taller, but those anterior to it are still undeveloped. The hypoglossal fissures are covered laterally only by dense mesenchyme. The

intrasclerotomic fissures have become obliterated, but their position is marked by a ring of less dense cartilage, anterior to the hypocentra. Except for the appearance of the metotic cartilages as faint mesenchymatous condensations on the otic capsules, there are few significant advances in this stage. The metotic fissure is still widely open.

The earliest indications of pleurocentral elements of the proatlas, atlas, and axis anlagen are found in the 9.2 mm. stage, though here they are not yet clearly differentiated from one another. The hypocentra of the atlas and axis remain independent, while those of the proatlas and other cervical vertebrae are already fused with the pleurocentra posterior to them. The hypocentrum of the 1st absorbed occipital vertebra (8th skeletal merome) is still evident but disappears before the next stage (cf. de Beer and Barrington, 1934, fig. 32). The acrochordal attains its maximum development in this stage. The flexures between the cervical and occipital regions, as well as those between the otic and acrochordal regions, which are already present in the 4 mm. stage, have become more pronounced. Likewise the vertical undulation of the chorda is now more exaggerated. The chorda is distinctly S-shaped (Fig. 2C) and anteriorly in two places lies very close to the dorsal surface of the basal plate. After approaching the dorsal or cerebral surface for the second time it comes to lie approximately midway between the dorsal and the ventral surfaces of the basal plate, and then rises gradually towards the dorsal surface in the occipital region.

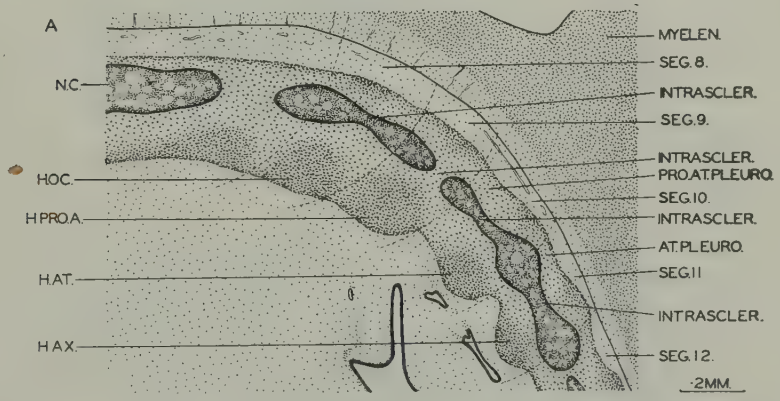
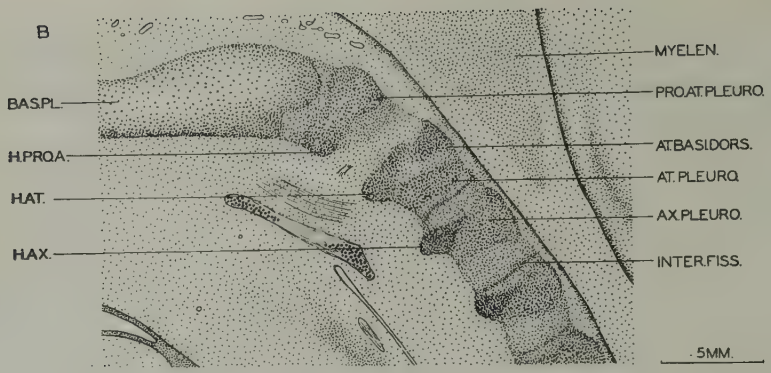


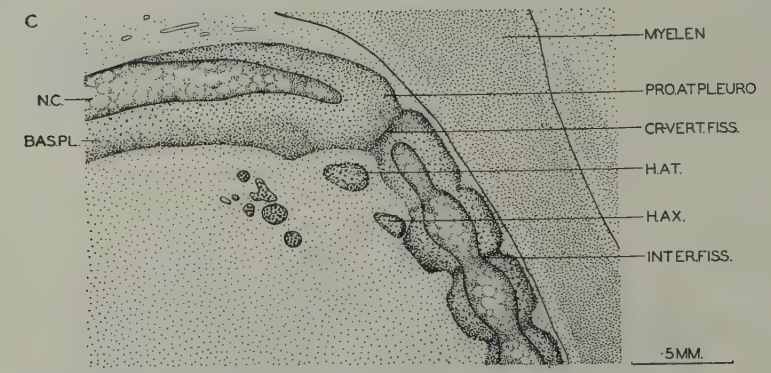
FIG. 3

Occipito-atlantic joint in the ostrich. Anterior end of sections to the left.

A .Sagittal section through 5.4 mm. embryo.



B. Parasagittal section through 10.7 mm. embryo.



C. Sagittal section through 11.0 mm. embryo.

D. Parasagittal section through 15.3 mm. embryo.

In a 10.7 mm. embryo, development has proceeded to a point where the atlantic pleurocentrum is losing its independence and is becoming attached to the axis, posterior to it, to form the processus odontoideus (Fig. 3B), while the pleurocentrum of the proatlas remains attached to the basal plate, eventually to form the occipital condyle. This differentiation and development of the vertebrae, as well as the appearance of deep intervertebral fissures in the perichordal tissue, is evident in a slightly older embryo (Fig. 3C). The components of the atlas, the paired basidorsals and the hypocentrum, are syndesmotically united, as in other cervical vertebrae (Fig. 3B). The last hypoglossal foramen has now been established as a result of the chondrification of the neural arch of the first absorbed cranial vertebra (basidorsal of 8th skeletal merome). These occipital arches, still precartilaginous at their distal extremities, are beginning to make contact over a large area with the precartilaginous medio-ventral edges of the otic capsules, thus closing the metotic fissure from behind. All that remains of the canal for the oculomotor nerve in the acrochordal cartilage is a shallow groove roofed over by connective tissue. A thinly chondrified prefacial commissure, lying antero-dorsally to the otic capsule, separates the facial foramen from the prootic fissure.

The anlagen of the metotic cartilages, visible from the 8.4 mm. stage (Fig. 13A) onward, appear to have separate centres of chondrification and for a very long time remain in a semi-precartilaginous state (Fig. 2A). Even in a 12.4 mm. embryo they appear as diffuse masses of lightly chondrified mesenchyme closely underlying the otic capsules posterior to the middle ear and extending caudally as far as the occipital region (Figs. 6B and 16A). Anteriorly they are completely fused along their dorsal edges with the ventral surfaces of the otic capsules. Here they form supports for the otic processes of the partes quadratae, being intercalated between these processes and the otic capsules. Their antero-ventral edges are still free; the cavum metoticum thus opens ventrally as well as anteriorly. Posteriorly the metotic fissures are closed because of an upgrowth of the occipital and precartilaginous preoccipital arches which rest against the otic capsules with their antero-dorsal edges. Laterally the occipital arches are indistinguishably fused with the metotic cartilages (Fig. 11). Nevertheless, medio-anteriorly they still leave a large portion of the metotic fissure open for the passage of the glossopharyngeal and vagal nerves. In this particular specimen there are three complete hypoglossal foramina on one side of the head, while on the other there are only two. The basal plate itself has undergone few significant changes beyond the expected expansion. Laterally, where it is confluent with the otic capsules (Fig. 4), it seems to contribute quite extensively to their medial cochlear walls, the suture joining the cochlear portions of the capsule to the basal plate being particularly broad and deep. (The question whether the basal plate actually forms part of the otic capsular wall will be dealt with later.) The acrochordal cartilage, which first appears in a 5.4 mm. embryo and reaches its maximum development in a 9.2 mm. embryo, thereafter slowly atrophies. In the 12.4 mm. embryo it appears as a small cartilaginous ridge on the dorsal edge of the basal plate, between the pilae antoticae and above the point where the tip of the chorda protrudes through the anterior surface of the dorsal plate (i.e. its ventral surface morphologically).

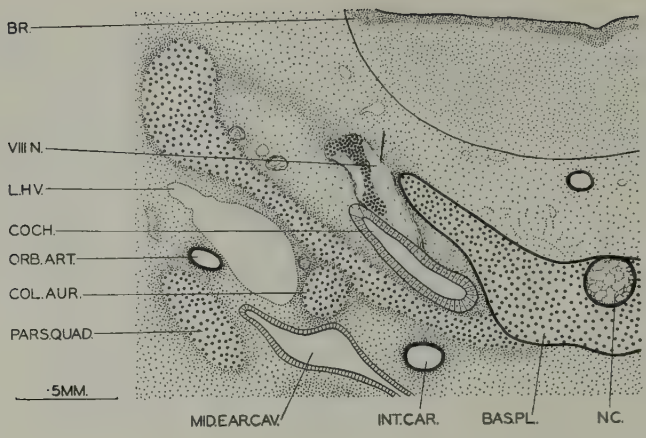


Fig. 4

Ostrich embryo 11.6 mm. Transverse section through the basal plate and otic capsule.

All that remains of the acrochordal in a 15.3 mm. embryo is a dense band of connective tissue which is attached laterally to the pilae antoticae and has its ventral edge free except for a point of attachment immediately above the chorda. The slightly hooked chorda tip consequently protrudes freely from the dorsal edge of the basal plate. The metotic fissure is now completely closed by the metotic cartilage, except at the recessus scalae tympani. The closely associated vagus and glossopharyngeal nerves leave the cranial cavity through a common foramen medial to the apertura medialis recessûs scalae tympani (Fig. 5). This foramen has been seperated from the reduced fissura metotica, by the growth around it of the basal plate and metotic cartilage (it is impossible to distinguish between the two). In this embryo, in contrast to the previous one, there are three pairs of hypoglossal foramina, and the oculomotor nerves pass freely over the dorsal edge of the reduced acrochordal. The presence of transitory anlagen of three cranial ribs found only in this stage is a point of special interest (Fig. 3 D). They are serially homologous with those of the cervical region. These ribs participate in the formation of the foramina transversaria for the passage of the vertebral artery. The cranial ribs as well as those of the cervical region are chiefly mesenchymatous with only the slightest cartilaginous matrix. They appear as small processes lying medio-ventral to the vertebral artery and lateral to the last absorbed occipital hypocentral element. They are in no way connected with the metotic cartilage lying dorso-lateral to them.

Soon after the 15.3 mm. stage the dermal bones of the skull make their appearance, and thereafter little change takes place in the chondrocranium. In an embryo of 17.7 mm. the occipital arches are interconnected by a precartilaginous tectum posterius, which is confluent with the precartilaginous tectum synoticum. By the time the head is 21 mm. long this connexion has chondrified and forms an arch between the otic capsules and between the occipital arches (Fig. 7B). Mesially they are completely fused, but laterally, where they are

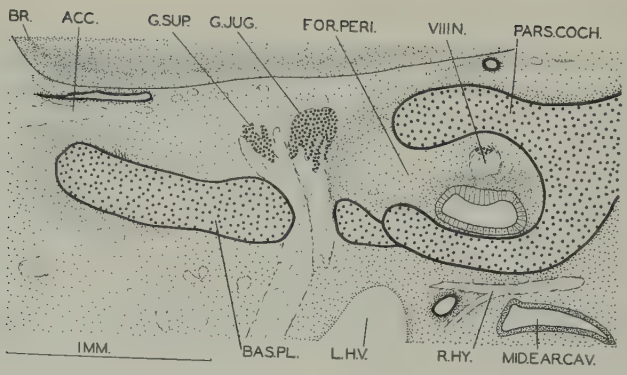


Fig. 5

Ostrich embryo 15.3 mm. Parasagittal section through basal plate and pars cochlearis. Anterior end of section to the right.

attached to the otic capsules and occipital arches respectively, the tectum synoticum and the t. posterius are separated by areas of incomplete chondrification. These vacuities close before the 21-day stage, but in the single 34-day stage specimen examined, one side is still open.

In the 21-day-old embryo the various components of the developing chondrocranium are becoming continuous. The metotic cartilage now fuses with the otic capsules at several points and is indistinguishably fused with the basal plate (Fig. 8B). The apertura medialis recessûs scalae tympani is almost closed by a downgrowth of capsular cartilage fusing with the basal plate. Only a small opening remains lateral to the internal opening of the common foramen for the passage of the glossopharyngeal and vagus nerves.

In the 34-day-old embryo separate foramina for the glossopharyngeal and vagus nerves have developed on one side of the head; on the other the nerves still pass, as in previous stages, through a common foramen. The external opening of the glossopharyngeal foramen lies anterior and lateral to that of the vagus, with the result that the nerve lies in the recessus scalae tympani and enters the brain cavity viá the apertura medialis recessûs scalae tympani. Although the internal opening of the glossopharyngeal foramen is confluent with that of the vagus, it is very wide and permits the vagus to enter directly into the cranial cavity without passing through the recessus scalae tympani. It is impossible to say what rôle, if any, the metotic cartilage may have played in the formation of these two foramina.

Although the number (3 pairs) of hypoglossal foramina appears to be constant in earlier stages it varies considerably in the older embryos. In a 21-day-old embryo examined, only two are present on one side while on the other side there are three. But the 34-day-old embryo has 3 foramina on either side of the head.

(ii) *Discussion.*

Contrary to conditions in all other described birds the acrochordal cartilage in the ostrich develops only some time after the perichordal plate has been laid down, the curved tip of the chorda projecting in early stages freely from its dorsal edge. Later, when the acrochordal is fully developed, it is pierced like that of the kestrel (Suschkin, 1899) by the oculomotor nerves.

In the 34-day-old ostrich embryo the connective tissue left after the recession of the acrochordal is not ossified, as is the case in the penguin (Crompton, 1953). It is possible, however, that it occurs during later development.

In a misquotation from de Beer and Barrington (1934), Crompton (1953) ascribes to them the coinage of the term perichordal plate for the unpaired parachordal anlage. However, since this new term is more descriptive than de Beer and Barrington's term "perichordal cartilage" (Umhüllingsmasse of Suschkin, 1899) and less likely to be confused with the terms suggested by Piiper (1928) for structures developed during the ontogenesis of the vertebral column (perichordal tube, perichordal rings) it is suggested that Crompton's term be retained in preference to that of de Beer and Barrington.

The S-shaped curvature of the perichordal plate described by Suschkin, (1899) in the kestrel also occurs in the ostrich. Lang (1952) in her work on cranial flexure of birds has termed this curvature "lordosis" of the basal region.

In contrast to the gull, *Larus* (Piiper, 1928), where the cervical perichordal rings are complete, those of the ostrich are open above i.e. below the neural tube. However, Piiper does observe that in the thoracic region the lateral portions of the perichordal rings are considerably thicker than the dorsal and ventral parts.

Sonies (1907) notes that the notochord takes a dorsal course through the perichordal plate in the fowl (as in the ostrich) but he does not record the condition in the duck, neither do de Beer and Barrington (1934) describe the position of the notochord in their work on the duck. The irregular horizontal curvature of the chorda in the occipital region of the basal plate, described and illustrated by Sonies in the fowl, was not found in the ostrich. Suschkin (1899) found only one dilatation of the chorda in the posterior region of the basal plate and this is probably the intersclerotomic dilatation belonging to the proatlas vertebra. Three dilatations presumably belonging to the last three absorbed occipital vertebrae are found in the basal plate of the ostrich. The basal plate of the emu (Lutz, 1942) both broadens and deepens in between the otic capsules in a way exactly similar to that of the ostrich. Lutz also mentions that in the emu there are no boundaries in the cartilage between the basal plate and the cochlear portions of the capsules. This is probably due, either to the total fusion of the basal plate with the cochlear portions of the otic capsules in the embryo he describes, or, as in the ostrich, to the apparent absence of a separate medial wall to the cochlear part of the capsule, its function being apparently taken over by the basal plate itself.

The investigation confirms Brock's (1937) findings but refutes de Beer's (1937) claim that a basiscranial fenestra occurs in the ostrich. Amongst birds investigated it is only the penguin, night-jar, emu and ostrich which lack a basicranial fenestra. Other groups in which this fenestra is absent are the

Dipnoi, Anura and Crocodilia (de Beer, 1937). In *Pyromelana* (Engelbrecht, in press) and the Kestrel (Suschkin, 1899) the basal plate is initially complete, a basicranial fenestra appearing subsequently as a result of absorption of the cartilage, much in the same way as in some *Urodela.* It is possible that the absence of a basicranial fenestra in some birds is a primitive feature, especially in view of the condition in the *Crocodilia, Anura* and *Dipnoi;* moreover in both the finch and kestrel the fenestra arises by absorption of cartilage in a formerly complete basal plate (and thus appears to be secondary). However, should the absence of a basicranial fenestra be considered a phylogenetically primitive condition, it is difficult to explain the reappearance in the duck and fowl of a fenestra which is present *ab initio,* as it is in most vertebrate groups.

When Suschkin (1899) claimed to have discovered cranial ribs lying between the myotomes of the head of the kestrel, Sonies (1907) criticized him on the grounds that it was doubtful whether one could identify myocommata as ribs before they were fully chondrified. Admittedly it is in these myocommata that ribs arise, but it seems premature, as Sonies says, to claim that these necessarily represent cranial ribs or even the anlagen of such ribs before any chondrification, however slight, has occurred, since there is nothing present to distinguish such a rib anlage from the ordinary mesenchyme of the myocomma. In a young embryo of *Pyromelana,* Engelbrecht (in press) found faint traces of intercellular substance between the cells of all three pairs of cranial myocommata, and it would seem legitimate to identify these as the anlagen of cranial ribs. Similarly in the duck, although there is no chondrification in the more anterior myocommata, de Beer and Barrington (1934, p. 424) record that "a small quantity of blue-stained intercellular substance is visible in the posterior two pairs". This also appears to be the case in the kestrel, as Suschkin (1899, p. 42) notes, "Der Charakter des Gewebes der Cranialrippen is derselbe, wie in den Rippen der vorderen Halsregion, doch ist das Gewebe weniger dicht." So it may be deduced that these "cranial ribs" are at least precartilaginous and not desmotic as Sonies thought. On the other hand Crompton (1953) claims that five cranial ribs (in a 43 mm. penguin embryo) "appear as areas of dense mesenchymatous tissue separating the myomeres below the perichordal plate". This stage was re-examined in Crompton's material, and although these areas exist they appear to be ordinary myocommata. (Even in a 56 mm. embryo there are no signs of chondrification here). What is more, these myocommata are in no way associated with their respective occipital hypocentra, as would be expected if they were rib anlagen (cf. the ontogenesis of cervical ribs). It may be mentioned here that four similar cranial myocommata are present in an ostrich embryo of approximately the same stage of development. Of the emu Lutz (1942, p. 322) says: "knorpelige Cranialrippen fehlen bei *Dromiceius*".

The cranial rib anlagen in the ostrich are attached to the basal plate and lie medio-ventral to the metotic cartilages in such a way as to make it impossible for them to contact the latter structures, much less give rise to them. In any case these rib anlagen appear long after the metotic cartilages are formed. Crompton (1953) has pointed out that in the penguin, where it is doubtful that cranial ribs are present, the position of the myocommata in which the ribs are reputed to arise rules out any possibility of their having given rise to the metotic cartilage. The earliest anlage of the metotic cartilage in *Pyromelana* lies anterior to the three cranial ribs. The association

of the metotic cartilage in the duck with the cranial ribs seems to be merely secondary, owing to the large size of the anterior cervical ribs e.g. well-developed atlas rib. The present investigation fully supports the findings of Crompton, who denies the origin of the metotic cartilage from the cranial ribs and asserts that the various ways in which this cartilage arises (see Sonies, Suschkin, de Beer and Barrington et al.) make it impossible to come to any definite conclusion about its origin.

It may be noted here that Crompton (1953, p. 79) is mistaken when he quotes Brock as saying that the metotic cartilage in the ostrich "appears as a tract of mesenchyme continuous with the dorsal edge of the auditory capsule". Brock (1937, p. 228) actually describes its position as being dorsally "continuous with the tissue of the canalicular otic capsule".

There is no antero-ventral process of the metotic cartilage of the ostrich or night-jar (see later) comparable with the subcapsular process described by de Beer and Barrington (1934) for the duck, or Engelbrecht (in press) for *Pyromelana*.

In the duck and fowl Sonies finds that the metotic cartilage encloses the vagus and glossopharyngeal nerves in separate foramina. However, de Beer and Barrington (1934, p. 434) more accurately describe the medial margin of the metotic cartilage in the duck, "approaching the lateral margin of the basal plate in the region between the glossopharyngeal and vagus nerves" where cartilaginous fusion takes place. Owing to the complete fusion of the basal plate with the metotic cartilage in the ostrich before the formation of the common vago-glossopharyngeal foramen, it is impossible to ascertain which constitutes the boundaries of the foramen, or which subdivides it in the 34-day-old embryo. Brock (1937, p. 232) notes that in a 15-day embryo of 21 mm. (the oldest studied by her) the glossopharyngeal and vagus nerves "pass back through closely adjacent foramina in the anterior margin of the metotic cartilage". No sign of the subdivision of this foramen was found in an embryo of exactly the same age and head-length sectioned during the present investigation. Even in an embryo 6 days older these nerves are still very closely associated and emerge through one foramen. Brock herself seems uncertain, for in the very next sentence she refutes her previous statement by saying: "This unusual common exit of these two nerves I have confirmed in each stage of the ostrich." In the present study it was only in an embryo of 34 days incubation that this subdivision became apparent on one side of the head. The meaning of this variation in development is unknown and may possibly have an individual and no phylogenetical significance.

Sonies (1907) finds that in both the fowl and the duck the neural arches arise from paired centres of chondrification which are independent of the pleurocentral (Wirbelkörper) anlagen. A similar condition exists in the ostrich, where the basidorsals which eventually form the neural arches are found to be in the synchondrotic continuity with their hypocentra. They are, however, not completely separated from their pleurocentra, but are easily distinguishable as they are far more heavily chondrified.

Examination of the basal plate and metotic cartilages of a South African night-jar embryo of roughly the same stage of development as a 21 mm. ostrich embryo revealed nothing of particular interest. Both structures are typically avian in appearance. Anteriorly the acrochordal has atrophied, leaving only a very low posterior wall to the hypophysial fenestra. The chorda

is completely enclosed and is beginning to disappear. There is no posterior basicranial fenestra. Laterally the cochlear part of the otic capsule is broadly fused with the lateral edges of the basal plate (Fig. 9B). There is a well-developed prefacial commissure that, as far as can be made out at this late stage of development, has become almost completely emancipated from the basal plate, and lies between the otic capsule posteriorly and the pila antotica spuria anteriorly. The vagus and glossopharyngeal nerves have each a separate foramen which seems to have been formed in the basal plate, since the metotic cartilage is not attached to the latter in this region but more posteriorly (Fig. 9C). There are two pairs of hypoglossal foramina. The metotic cartilage has a very typical topography: its distal dorsal edge has become folded under a lateral extension of the otic capsule formed by the lateral semicircular canal in such a way as to form a concha-like structure with its opening facing into the middle ear cavity. Anteriorly this folded portion supports the otic process of the pars quadrata. There is a pair of well-developed occipital arches incompletely fused latero-anteriorly with the otic capsules (Fig. 9D). They appear to meet above the foramen magnum to form a tectum posterius, although it is possible that the cartilaginous arch connecting them may actually be part of the tectum synoticum lying immediately anterior to them.

(b) *THE OTIC CAPSULES*

(i) *Description.*

Because the auditory and especially the equilibrial functions of the internal ear in terrestrial vertebrates remain relatively unaffected by even the most far-reaching changes in environmental conditions, little of phylogenetic interest can be expected from a study of the development of the organs concerned in any particular animal.

In the ostrich, except for a few very general trends characteristic of the chondrocranial development as a whole, no unusual or distinguishing characteristics have been found. An example of these general trends is the tendency for the components of the otic capsules to develop *ab initio* in homocontinuity with themselves and the rest of the chondrocranium, rather than in heterocontinuity as seen typically in the development of the duck.

It is not until a head-length of 6 mm. is attained in ostrich embryos that there are any definite signs of chondrification in the region of the otic vesicle. But in a 5.4 mm. embryo the basal plate deepens between the vesicles to form the dorsomedial walls to the cochlear portions of the otic capsules (Fig. 12A and B). The mesenchyme surrounding these vesicles becomes slightly denser ventrally as well as laterally. In this stage the membranous otic vesicle is still relatively undifferentiated, having only a well-developed endolymphatic duct on its dorsal surface (Fig. 1).

By the time the head of the embryo is 6.8 mm. long the cochlear and canalicular portions of the membranous labyrinth are distinct and the endolymphatic duct has also grown much longer. Medially the cochlea is embedded in a shallow cup-like depression in the side of the basal plate that extends laterally in a broad precartilaginous process to form a postero-ventral floor to this portion of the labyrinth. The early anlage of the proximal part of the stapes is separated from this part of the otic capsule by means of

a layer of flattened mesenchyme cells bounding the two entities. The posterior wall of the capsule overlooking the metotic fissure in which the perilymphatic foramen will later differentiate, consists of dense mesenchyme, except for a short blunt pre-cartilagious process projecting laterally from the dorsal edge of the cup in the basal plate enclosing the cochlea. This is the dorsal cochleo-canalicular commissure (Crompton, 1953) as yet incompletely developed. Later it forms the dorsal border to the perilymphatic foramen. Anteriorly a broad precartilaginous projection of the basal plate immediately dorsal to the floor of the cochlear part of the otic capsule represents the early anlage of the more ventral part of the processus lateralis partis cochlearis (Crompton, 1953). The rest of the capsule consists almost entirely of dense mesenchyme passing over into the more precartilaginous parts without definite boundaries. Dorsally there is no roof to the capsule, except in the immediate vicinity of the endolymphatic duct where there is a slight thickening of the mesenchyme. Lateral to the processus lateralis partis cochlearis and around the facial nerve and geniculate ganglion, there is a large tract entirely devoid of precartilage and only thinly mesenchymatous.

In the 8.4 mm. embryo the cup-like depression containing the cochlea has become deeper because its walls have grown, while the precartilaginous floor extending laterally from the basal plate below the otic vesicle has increased in area and become trough-shaped. Anteriorly the processus lateralis partis cochlearis has fused with the canalicular part of the otic capsule ventral to the facial nerve (Fig. 13A). In this stage there is no fusion of the process dorsal to the nerve. This process is, however, still separated by a narrow unchondrified fissure from the above-mentioned floor of the capsule. The position of the perilymphatic foramen is marked by an area of dense mesenchyme devoid of any cartilage matrix. Its dorsal border, formed by the dorsal cochleo-canalicular commissure is now complete, the commissure having fused with the canalicular portion of the otic capsule lateral to the foramen (Fig. 13B). With the fusion of the processus lateralis partis cochlearis with the canalicular part of the otic capsule the pro-otic fissure is completed posteriorly. Similarly the anterior border of the metotic fissure has become delimited by the growth and fusion of the dorsal cochleo-canalicular commissure with the canalicular capsule. The stapes and capsular wall are now more clearly discontinuous owing to progress in chondrification. There is, however, no indication of a fenestra ovalis. It may be especially noted from transverse sections through this region that the side of the capsule nearest the brain, the roof, is still completely undeveloped, while most of the more lateral and ventral parts of the capsule consist of either dense mesenchyme or precartilage. This unchondrified dorsomesial part of the capsule must represent the cochleo-canalicular fissure, the more ventral part of which has become obliterated by the precocious development of the capsular floor. (See discussion).

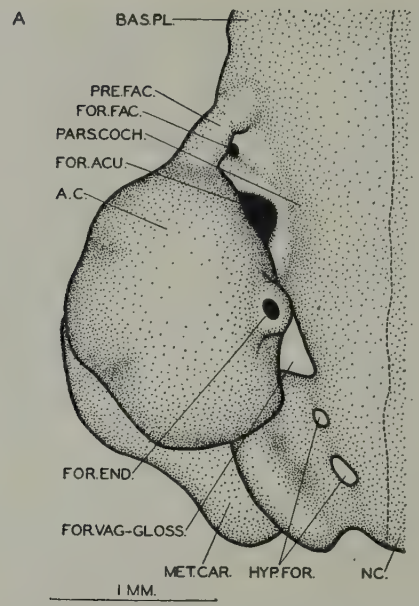
Although the chondrification of the otic capsule of a 9.2 mm. embryo is more advanced than that of the previous stage it shows no significant changes. However, elements present in the previous stage are now more heavily chondrified and consequently more easily observable (Fig. 2B). The facial nerve, after leaving the geniculate ganglion lying dorsal to the cochlear portion of the capsule, proceeds in a antero-ventral direction over the processus lateralis partis cochlearis (Fig. 10).

The main advance in the 10.7 mm. stage is medially, in the roof of the cochlear portion of the capsule: it is now more heavily chondrified than the floor which has remained much as it was in previous stages where it was the more advanced of the two. This acceleration in the chondrification of the cochlear roof creates the impression that this portion of the capsule is derived from the basal plate, whose chondrification at this time is ahead of the rest in this region (Fig. 4). It may be noted at this point that the cochlear portions of the otic capsules are almost horizontal and underlie the basal plate. It is only the more lateral canalicular portions which are more or less vertically placed in relation to the basal plate. With the progressive chondrification of the roof of the capsule a wide common foramen has become delimited medially; it represents a remnant of the dorsal part of the cochleo-canalicular fissure for the branches of the auditory nerve and endolymphatic duct. Laterally, however, it is still confluent with the large unchondrified area in the roof of the canalicular portion of the capsule. Anteriorly and posteriorly it is bordered by the processus lateralis partis cochlearis and the dorsal cochleo-canalicular commissure respectively. Antero-dorsal to the processus lateralis partis cochlearis a thinly chondrified prefacial commissure has developed, which separates the facial foramen from the prootic fissure. Laterally the commissure is attached to the canalicular portion of the capsule, while medially it appears to be connected with both the basal plate and the cochlear capsule. Since these two entities are indistinguishably fused, their limits can only be approximately determined. However, the cochlear sac is comparatively small in the ostrich and its enclosing cartilage can give only partial support to the prefacial commissure (Fig. 10). The more lateral walls and floor of the canalicular part of the otic capsule, in contrast to the roof, are in an advanced precartilaginous condition. That portion of the capsular wall against which the proximal part of the stapes abuts is becoming separated from the surrounding wall by disintegration of the cartilage immediately surrounding it.

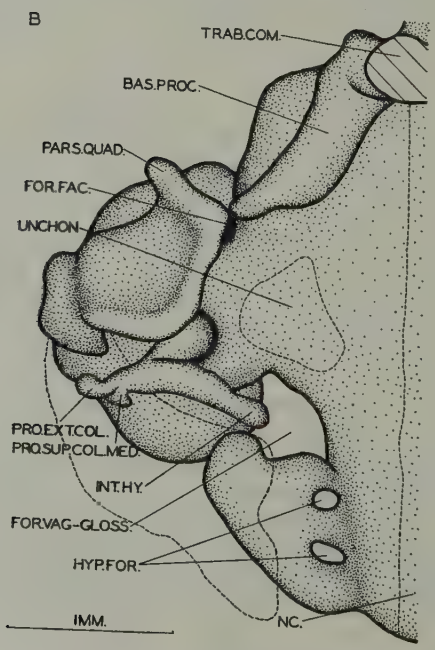
The antero-dorsal edges of the precartilaginous occipital and preoccipital arches have come into contact with the ventral surface of the otic capsules in an embryo of 11.6 mm., thus closing the metotic fissure posteriorly. This latter is now delimited posteriorly by the growing occipital arches, dorso-laterally by the ventral edge of the otic capsules and ventro-medially by the basal plate. Into its most anterior part opens the perilymphatic foramen, the dorsal rim of which (i.e. the dorsal cochleo-canalicular commissure) is as heavily chondrified as the basal plate and continuous with it, while the rest of the capsule in this region is still precartilaginous. The impression is thus created that the rim is formed by lateral projections of the basal plate. The metotic cartilages underlie the otic capsules but are not yet attached to them.

In the 12.4 mm. stage the otic capsule is beginning to take on its definitive form (Fig. 16A and B). Most of it is chondrified but the medial wall of the canalicular portion of the capsule is still only thinly mesenchymatous. Anteriorly there is a heavily chondrified prefacial commissure lying between the basal plate and cochlear capsule medially, and the canalicular portion of the otic capsule laterally (Fig. 6A). The cochlear part of the capsule is still only slightly chondrified ventrally (Fig. 6B). The cartilage in the foramen ovale has now almost completely atrophied, leaving the proximal part of the

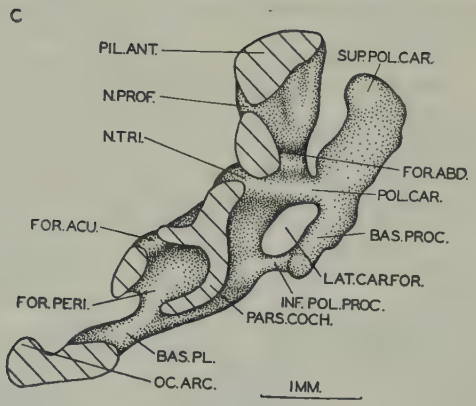
FIG. 6: Ostrich embryo 12.3 mm.



A. Dorsal reconstruction of basal plate and otic capsule. The mesenchymatous median wall of the otic capsule has been included.



B. Ventral reconstruction of basal plate and columella auris. The outline of the metotic cartilage is indicated by dotted lines.



C. Lateral reconstruction of portion of chondrocranium to show disposition of otic capsule and related entities.

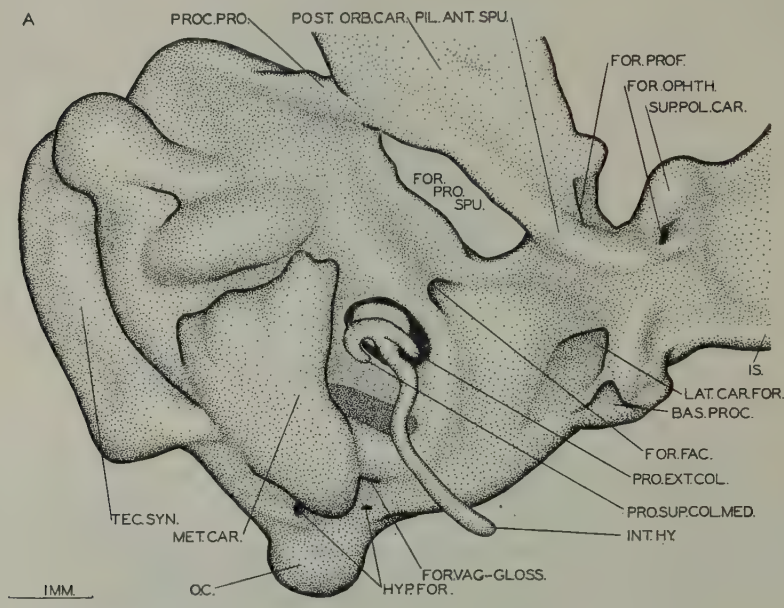
stapes to fill the opening. All but the most anterior part of the metotic fissure is closed by the continued upgrowth of the occipital arches against the otic capsules. The metotic cartilage which is closely applied to the ventral aspect of the capsule is indistinguishably fused with the occipital arches, thus closing the fissure laterally. The perilymphatic foramen opens into this remnant of the metotic fissure (viz. the recessus scalae tympani) and through it pass the vagus and glossopharyngeal nerves (Fig. 11).

The sides of the otic capsules facing the brain consist of thin precartilaginous material in a 15.3 mm. embryo (Fig. 17A and B); there is, however, still a large common foramen for the acustic nerve and endolymphatic duct. A deep indentation, the fossa subarcuata, has appeared in the capsular wall immediately below and medial to the upper semi-circular canal. The metotic cartilage is becoming more densely chondrified, and dorsally a portion of it has grown in between the otic process of the pars quadrata and the otic capsule.

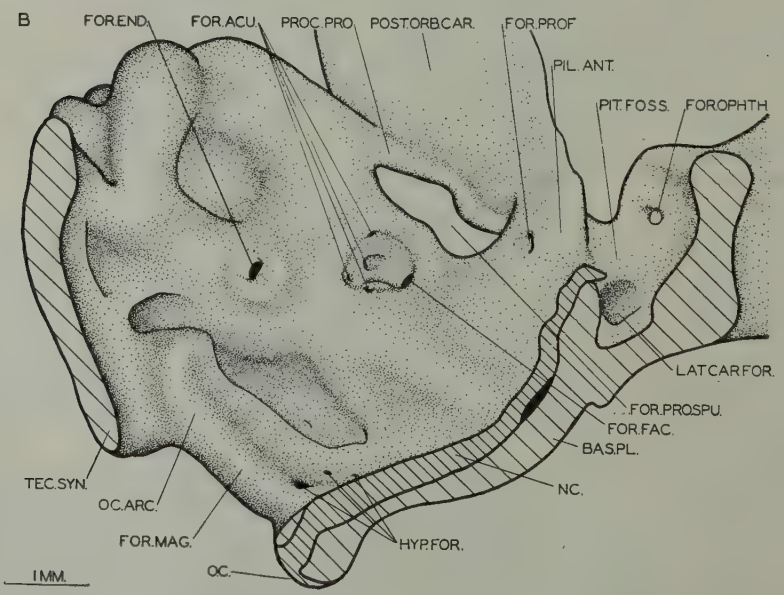
Shortly after the above stage (15.5 mm.) the medial wall of the capsule becomes completely chondrified except for a small opening continuous with and ventral to the endolymphatic foramen. There is also a narrow unchondrified tract between the cartilage surrounding the upper semicircular canal and the rest of the capsule. The floor of the cochlear portion of the capsule is fully chondrified except for a small precartilaginous area. A slight depression in the median wall of the capsule anterior to the opening for the endolymphatic duct, lodges the acustico-facial ganglion. In this depression five acustic foramina may be found.

Now that the otic capsules are taking on their definitive form very few changes occur. In the 17.7 mm. embryo a precartilaginous arch appears posteriorly, stretching between the two capsules as well as between the occipital arches. Its actual extent is difficult to ascertain owing to its diffuse character, but it would seem that a tectum posterius is well developed, while anteriorly an incomplete tectum synoticum is fused with it. In some specimens the two tecti are separated by small unchondrified tracts at their bases.

Antero-dorsally on each capsule a ridge of dense connective tissue has appeared connecting the posterior orbital cartilage to the otic capsule of the same side. Its morphology is discussed below.



A. Lateral View.



B. Median View.

Fig. 7

Ostrich embryo 21.0 mm. Reconstruction of otic capsule. Anterior region of chondrocranium omitted.

In the 21 mm. embryo the capsular walls are fully chondrified, closely surrounding the various membranous structures they support (Fig. 7A and B). The shape of the membranous labyrinth is reflected in the several prominences and depressions exhibited externally by the capsule. With the growth of the cochlear portion of the capsule the prefacial commissure has come to lie completely dorsal to it, between the more postero-dorsal portions of the otic capsule and the base of the pila antotica spuria. Dorsal and posterior to the prefacial commissure the posterior orbital cartilage has now fused with the low, laterally directed ridge, the "prootic process" (de Beer and Barrington, 1934), on the dorsal edge of the capsule. Previous to this stage the otic process of the pars quadrata has been supported entirely by the metotic cartilage which has been intercalated between the process and the capsule. In this stage the otic process is supported medially by the capsule and laterally by the metotic cartilage. In a slightly older embryo (21 days) the capsule develops a ridge laterally, the crista parotica; this bears against the medial face of the otic process (Fig. 8B). The latter now articulates laterally with the metotic cartilage, medially with the capsule itself and slightly anteriorly also with the crista parotica. The metotic cartilage is as yet imperfectly attached to the capsule for the boundaries of the two structures still retain their identity. The fused tectum synoticum plus posterius is in the form of

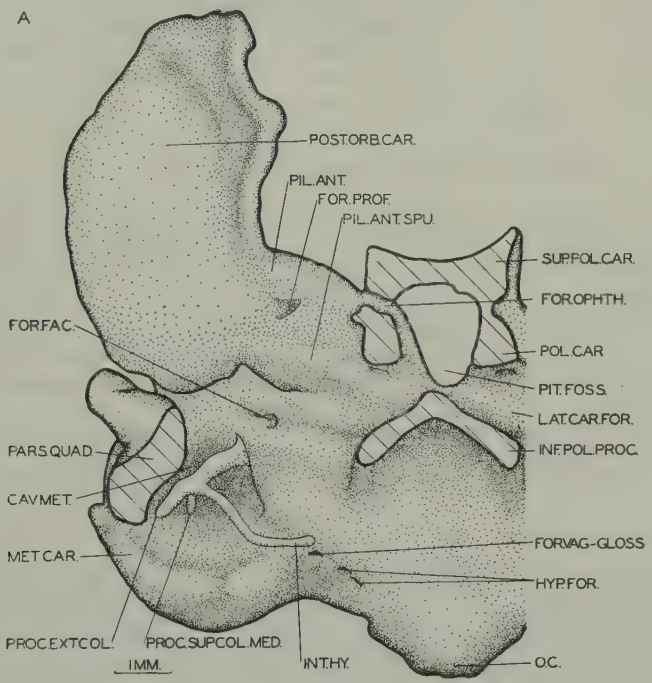
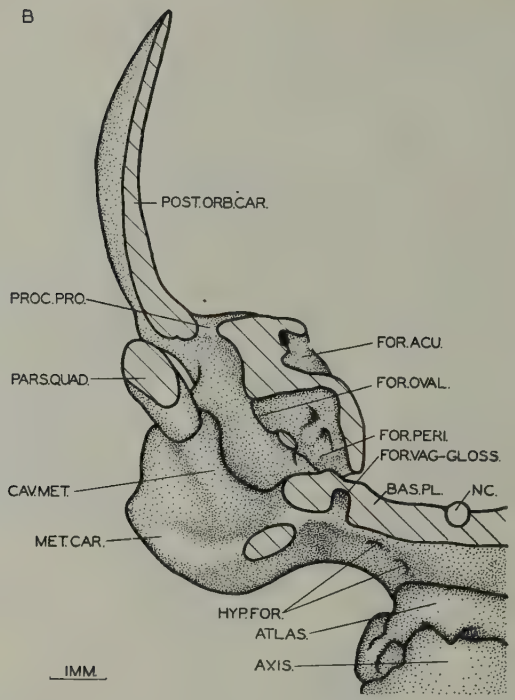


Fig. 8

Ostrich embryo 21 days.
A. Anterior reconstruction of posterior region of chondrocranium.



B. Reconstruction of basal plate and otic capsule.

a high vertical plate lying above the foramen magnum, between the otic capsules and the occipital arches on each side and closing the chondrocranial cavity from behind. The foramen for the endolymphatic duct is situated on a small prominence on the medial wall of the capsule. The footplate of the stapes is now attached by strong connective tissue, in which isolated cartilage cells are still visible, to the rim of the foramen ovale. The foramen perilymphaticum lies ventral to the foramen ovale and faces into the deep cavum metoticum.

After the stage described above no changes could be observed in the otic capsules until the embryo was 34 days old and ossification set in ventrally.

(ii) *Discussion*

The relations of the otic capsules to the basal plate in higher amniotes have become complicated by the encroachment of the basal plate area by the cochlear part of the capsules. So much has this taken place in most birds and higher mammals that the structures concerned almost meet each other in the midline. Nevertheless, the avian basal plate is not a composite structure as Crompton (1953) suggests, since in most birds (and mammals) the cochlear capsules are initially discrete and not partly formed by the basal plate. De Beer (1937, p. 401), who has already dealt exhaustively with the

problem, comes to this conclusion: "there can . . . be no doubt that the cochlear capsules are extensions of the true auditory capsules which displace the antero-lateral parts of the basal plate, but are in no way formed out of it". In this region, therefore, the lateral border of the avian basal plate skirts the limits of the cochlear capsule as it does in *Lacerta,* in which the cochlear capsules are small. It then turns laterally forming the ventral edge to the prootic foramen.

In lower vertebrates the prootic and the metotic fissures lie between the lateral edge of the basal plate and the medial border of the capsule, but with the expansion of the cochlear capsule into the basal plate region, the lateral edge of the latter lies nearer the canalicular portion of the capsule, and the prootic and the metotic fissures come to lie directly in line with the cochleo-canalicular fissure. This relative shifting has necessitated a similar change in the position of the avian prefacial commissure which now lies dorsal instead of medial to the otic capsule. In some extreme cases, such as the penguin (Crompton, 1953) and *Pyromelana* (Engelbrecht, in press) and perhaps in the duck (Sonies, 1907), the basal plate seems to have been totally excluded from support of the commissure by the cochlear capsule, the former coming to lie between the two parts of the otic capsule as it does in mammals. However, in the penguin there is no certainty on this point, as the cochlear capsule fuses completely with the basal plate before the commissure becomes continuous with the canalicular portion of the otic capsule. In the ostrich, unfortunately, the cochlear capsule is small and fused with the basal plate from the earliest stages, so that it is impossible to ascertain its actual extent. Gray (1906 and 1908) in his studies on the membranous labyrinth maintains that the cochlea of the *Ratitae* in general is relatively small. It is thus clear that the basal plate at least partially supports the prefacial commissure anteriorly while its proximal posterior portion is continuous with the cochlear part of the capsule. Conditions in the ostrich are thus transitional and support the theory of Gaupp (1906), Sonies (1907), and others that the prefacial (suprafacial) commissure as found in mammals and birds is homologous with that of the lower amniotes.

Although as early as 1907 Sonies observed the separate origin of the canalicular and cochlear portions of the otic capsules it was not until very recently that the several commissures joining the two entities during later development were described in any detail (see Crompton, 1953). De Beer and Barrington (1934) mention a strand of tissue marking the anterior limit of the metotic fissure in the duck embryo; judging from their description it apparently corresponds to the cochleo-canalicular commissure of the penguin. Later in the development the posterior margin of the prootic fissure becomes delimited by what appears to resemble the processus lateralis partis cochlearis of the penguin. But, whereas in the ostrich and duck the processus lateralis partis cochlearis develops completely ventral to the facial nerve (the prefacial commissure appearing only later in the ontogeny), in the penguin the facial nerve comes to lie between the distal end of the process and the canalicular part of the capsule in such a way as to be enclosed in a foramen. Crompton homologizes that part of the process lying antero-dorsal to the facial nerve of the penguin with the prefacial commissure of the duck, so that the part lying postero-ventrally to the nerve must be homologous with the process as it is found in the ostrich and duck.

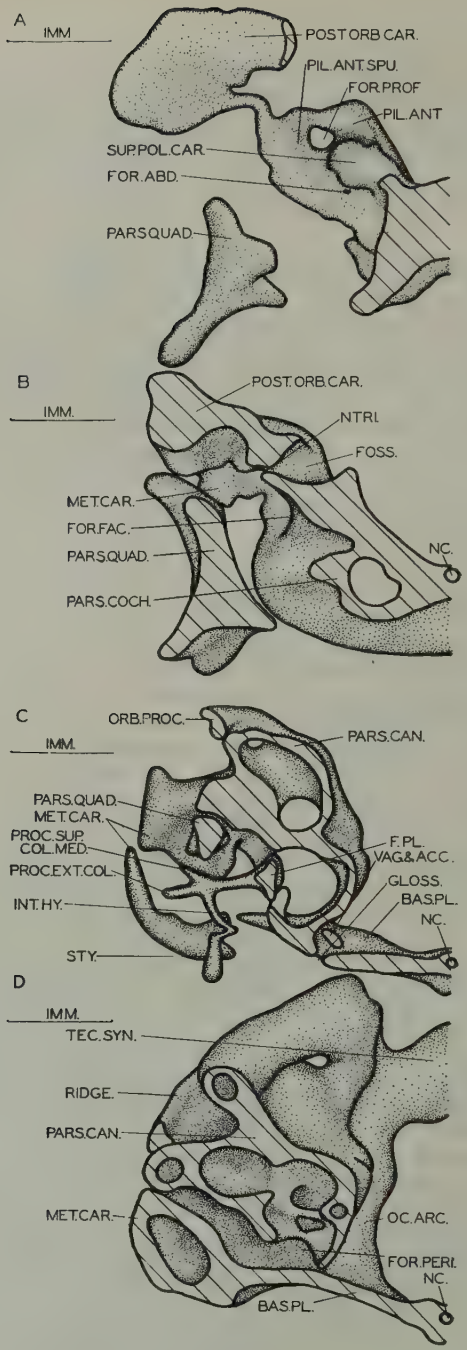


Fig. 9

Night-jar embryo. A series of stereograms of the posterior region of the chondrocranium.

Note: In C. the processus supracolumellaris lateralis is hidden behind the processus supracolumellaris medialis.

The early ontogeny of the otic capsules of the ostrich follows exactly the general description given by Gaupp (1906, p. 801) on the development of the chondrocranium of birds viz., "Lateralwärts geht . . . das Gewebe der Basalplattenanlage kontinuierlich in das periotische Gewebe über, und auch die Verknorpelung schreitet von der Basalplatte aus auf des letztgenannte Gewebe vor, so dass auf jüngeren Stadien die Ohrkapsel eine mit der Basalplatte zuzammenhängende, oben offene Schale darstellt". But this statement does not necessarily imply, as de Beer seems to think it does, that Gaupp considered the cochlear part of the capsule in birds to be a derivative of the parachordals! The observations of de Beer and Barrington (1934) on the duck, and of Crompton (1953) on the penguin, inexorably point to the conclusion to which Gaupp came in 1906, that this continuity in development is purely secondary. Nevertheless the subsequent development of the otic capsules of the ostrich seems to indicate that part of the cochlear capsule (the roof) is formed by the basal plate. For although the basal plate and neighbouring structures are equally well developed in earlier stages, as the ontogeny progresses the chondrification of the basal plate precedes that of the rest of the chondrocranium, and with it that of the roof of the cochlear part of the capsule. This gives the impression that the cochlear part of the capsule is a derivative of the perichordal cartilage, since the rest of the capsule is still mainly precartilaginous. The same conditions of homocontinuous development exist in the kestrel (Suschkin, 1899) and *Pyromelana* (Engelbrecht, in press). These are probably phenomena brought about by heterochrony, having no morphological significance; nevertheless it is interesting to note that Crompton (1953) observes that "all that now remains (stage III) to indicate the presence of the partes cochleares are two shallow grooves in the ventral surface of the basal plate. The grooves lodge the cochlear sacs of either side." The conditions in the penguin are thus exactly similar to those of an 11 mm. ostrich embryo, except that in the ostrich a thin precartilaginous floor is already present in the cochlear part of the capsule whereas in the penguin this floor develops much later. Dorsally in the ostrich, as in the penguin and *Pyromelana*, a remnant of the cochleocanalicular fissure persists as the acustic foramen.

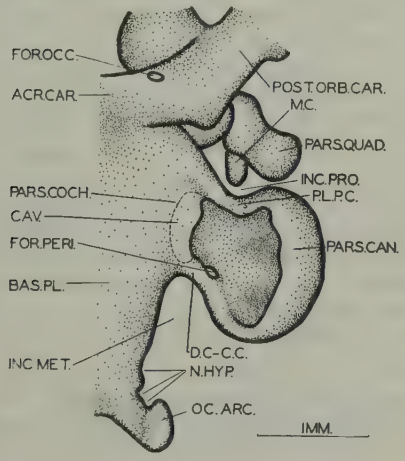


Fig. 10

Ostrich embryo 9.2 mm. Dorsal reconstruction of posterior region of chondrocranium.

Crompton (1953) states that in the young penguin embryo the facial nerve has to pass up through the cavity of the capsule in order to reach the geniculate ganglion. It is difficult to see what he means by this statement, since, as the capsular roof is not yet formed mesially in this stage, it is impossible to ascertain whether it lies within or without the boundaries of the capsule. From dorsal reconstructions it appears to lie within the capsular limits, but this is apparent and not real, for with further development a cartilaginous wall appears below the nerve, roofing the capsule in this region and excluding the nerve from its cavity. There is a similar delay in the chondrification of the roof of the otic capsule in the ostrich and finch. The relation between the facial nerve and otic capsule throughout the vertebrates has been fully dealt with by de Beer (1937).

As the ventral portion of the cochleo-canalicular fissure is obliterated from the earliest stages of the development of the ostrich, no additional light was brought to bear on Sonies's (1907) claim that the fenestra ovalis is a remnant of this fissure. De Beer and Barrington (1934, p. 430) describe the position of the stapes thus: "laterally to the cochlear capsule, the rudiment of the columella auris can be seen as an isolated structure." This is not very clear but it does seem to corroborate Sonies's statement. In the penguin, however, although there is a well-developed cochleo-canalicular fissure the stapes is fused with the canalicular portion of the capsule (Crompton, 1953). In both the ostrich and *Pyromelana* (Engelbrecht, in press) the stapes anlage is fused with the floor of the otic capsule.

The otic capsules of the South African night-jar embryo (corresponding in development to a 21 mm. ostrich embryo) are typically developed. The prominentia semicircularis lateralis projects laterally farther than it does in the ostrich with the result that the otic process of the pars quadrata, and especially by the metotic cartilage, instead of lying lateroventral, as in other birds, is tucked beneath it (Fig. 9B en C). On its dorsal edge a high ridge of precartilage appears which runs posteriorly to meet the cartilage surrounding the upper semicircular canal at its most caudal point (Fig. 9D). During the development of the otic capsules of the ostrich, precartilage fills the groove between the cartilage surrounding the lateral and upper semicircular canals, but it is not elevated into a ridge. In common with other birds there is a shallow depression on the inner side of each capsule for the accommodation of the acustico-facial ganglia. Into this depression open the foramina for the branches of the acustic and facial nerves. Posteriorly the fossa subarcuata penetrates right through to the lateral surface of the capsule as it does in the water-rat, *Microtus,* and the bat, *Miniopterus* (de Beer, 1937). The capsules are joined above the foramen magnum by the tectum synoticum, which is indistinguishably fused with the tectum posterius medio-dorsally. Laterally, however, the two tecti are separated by a pair of foramina, as in certain stages in the development of the ostrich. Immediately anterior to the facial foramen there are two small areas of incomplete chondrification in the wall of the capsule, the one dorsal and the other ventral. Their exact significance is unknown but their presence suggests that the two parts of the capsule which they demarcate each has a separate origin (cf. other birds) and that these openings are the remains of the cochleo-canalicular fissure.

Although the prootic process (de Beer and Barrington, 1934) in the advanced ostrich embryo is in the form of a low ridge on the dorsal edge

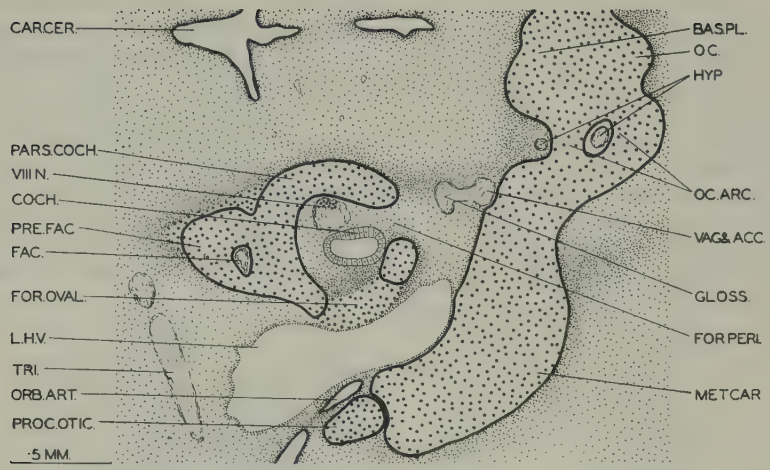


Fig. 11

Ostrich embryo 12.3 mm. Frontal section through basal plate and metotic cartilage. Anterior end of section to the left.

of the otic capsule, it is nevertheless in extended suture with the posterior orbital cartilage and completes the foramen prooticum spurium. This is probably due to the extreme proximity of the otic capsules to the posterior orbital cartilages which have been displaced caudally by the large eyes. In contrast the night-jar has a well-developed posteriorly directed process of the orbital cartilage, the orbito-capsular process (de Beer and Barrington, 1934) (processus posterior or oticus of Sonies, 1907), making a long antero-posterior suture with the dorsal edge of the otic capsule and closing the foramen prooticum spurium in much the same way as in the penguin (Crompton, 1953). The condition in the duck according to de Beer and Barrington (1934) is intermediate, both processes being developed but remaining unfused, with the result that the lateral margin to the foramen prooticum spurium is left incomplete. On the other hand Sonies (1907) describes the fusion of these processes and the formation of a foramen in both the duck and fowl.

In his concluding remarks on the ostrich chondrocranium de Beer (1937, p. 287) notes that "the taenia marginalis connects the posterior orbital cartilage with the auditory capsule." This taenia is obviously the same structure as that described here as the prootic process. Quite possibly the connexion between the posterior orbital cartilage and the otic capsule in birds is homologous with the taenia marginalis of the lizard as de Beer suggests; it certainly bears the same relations to both the nerves and the rest of the chondrocranium.

(c) *THE ORBITAL CARTILAGES AND TRABECULAE*

(i) *Description.*

The trabeculo-polar cartilages first appear in a 5.4 mm. embryo ostrich as a pair of faint precartilaginous structures attached to the perichordal

plate, latero-ventral to the point where the chorda projects from its anterior surface (Fig. 12A and B). They are distinctly paired and no connexion exists between them at this stage. Also, no separate polar cartilage anlagen or indications of them were found. Antero-dorsal to the point where the trabeculo-polar cartilage is fused with the perichordal plate, a relatively large but diffuse supra-polar cartilage is attached by thin precartilage posterior to the ophthalmic artery. Its posterior border is clearly indicated by a concentration of dense fibres. The short pilae antoticae which are mesially delimited by the canals for the oculomotor nerves are continuous with the acrochordal cartilage and project laterally from it. The abducens leaves the cranial cavity lateral to the pila antotica and is not yet enclosed by cartilage.

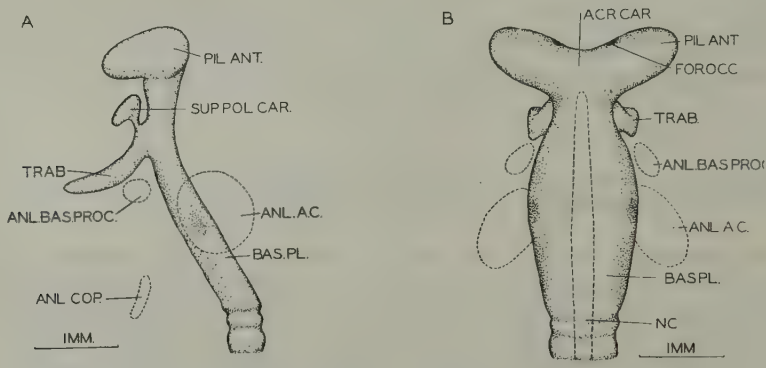


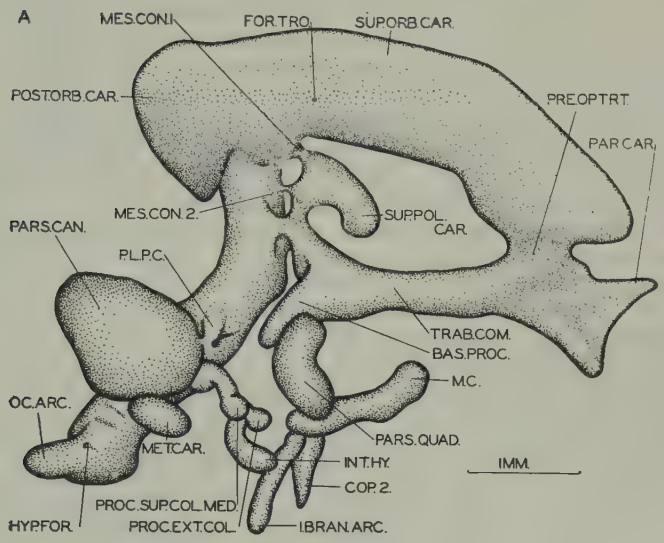
FIG. 12: Ostrich embryo 5.4 mm.

A. Lateral reconstruction of chondrocranium.
B. Dorsal reconstruction of chondrocranium.

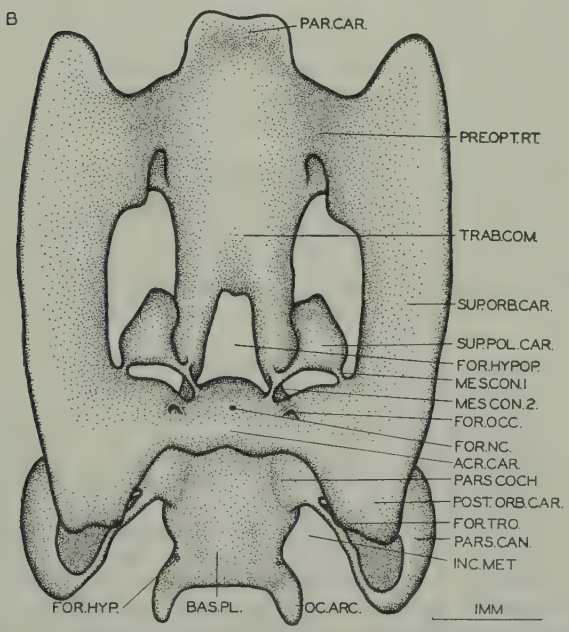
The matrix of the trabeculo-polar cartilages has become considerably denser in the 6.8 mm. stage. The suprapolar cartilage is larger, its connexion with the trabeculo-polar cartilage remaining densely fibrous. Dorsally each suprapolar cartilage is attached by means of thin precartilage to the pila antotica of its side, that is lateral to the oculomotor canal. The internal carotid or cerebral arteries enter the cranial cavity through the lateral carotid incisures, which are bordered anteriorly by the posterior edges of the trabeculo-polar cartilages, dorsally by the connexion of the latter with the basal plate, and posteriorly by the basal plate itself. The hypophysial incisure has as yet no anterior border as the trabeculae are not yet fused. Lateral to the posterior edge of the trabeculae and immediately dorsal to the palatine branches of the facial nerves the basitrabecular processes have arisen as independent mesenchymatous anlagen. They are actually already faintly discernible in a 5.4 mm. embryo. The earliest anlagen of the orbital cartilages are present as slight antero-lateral projections of the pilae antoticae.

The trabeculae first become connected anteriorly by a mesenchymatous proliferation between them in a 7.8 mm. embryo, thus the hypophysial incisure becomes a fenestra. The still independent mesenchymatous basitrabecular process now actually touches the dorsal edge of the anlage of the pars quadrata.

FIG. 13: Ostrich embryo 8.4 mm.



A. Lateral reconstruction of chondrocranium.



B. Dorsal reconstruction of chondrocranium.

The suprapolar cartilages have become attached to the acrochordal slightly ventro-laterally to the chorda tip by means of precartilaginous processes. More laterally may be discerned the very thinly chondrified commissure which attaches the dorsal edge of the suprapolar cartilage to the base of the pila antotica lateral to the canal for the oculomotor nerve. Although both these commissures are better developed in a slightly older embryo (Fig. 13A and B), they are transitory and soon disappear. The orbital cartilages appear in this stage as precartilaginous, anterior projections of the pilae antoticae and are still unattached anteriorly. The anterior roots of the orbital cartilages are represented by small processes on the trabeculae.

By the time the 8.4 mm. stage is reached the trabeculae have fused completely to form a trabecula communis (Fig. 13A and B). But, although they are fused, transverse sections through this region still reveal its double origin, for the trabeculae are more heavily chondrified than the cartilage which surrounds them. The densely mesenchymatous basitrabecular processes are becoming attached by mesenchyme to the trabecula communis. Their distal extensions lie dorsal to the anlagen of the pars quadrata. A small infrapolar process, which does not meet the basal plate, is present on one side of the head. The orbital cartilages are now continuous and consist of diffuse cartilage matrix in which the cells are widely spaced. Posteriorly the cartilages are broadly attached to the pilae antoticae, and as a result of their growth the trochlear nerves have become enclosed in long horizontal canals latero-dorsal to the oculomotor canals. Anteriorly the orbital cartilages are attached to the trabecula communis at a very obtuse angle posterior to the developing nasal sacs,

Contrary to conditions in the embryo just described, there are no signs of an infrapolar process in the 9.2 mm. embryo; there are, however, slight cartilaginous protuberances on the basal plate ventral to the internal carotid arteries at the point where the infrapolar cartilages join it in older embryos (Fig. 2A, B and C). The blasteme of the orbital cartilage has a slightly better developed matrix.

In a 10.7 mm. embryo the interorbital septum has made its appearance as a dorsal outgrowth of the posterior part of the trabecula communis, while anteriorly the orbital cartilages are attached over a wider area to the dorsal edge of the trabecula communis. Antero-dorsally the orbital cartilage has a small window. The posterior roots of the orbital cartilages (pilae antoticae) have increased in height, thus displacing the orbital cartilages further above the eyes.

In the 11.0 mm. embryo the suprapolar cartilage has increased in size and, both anterior and posterior to the ophthalmic artery, is fused with the trabeculo-polar cartilage. The commissure between the suprapolar cartilage and the acrochordal has broken down. But it should be noted that in a slightly larger embryo (11.6 mm.) this commissure is still present on one side of the head; it is absent in all older embryos. The dorsal process of the suprapolar cartilage attaching it to the pila antotica immediately lateral to the exit of the oculomotor nerve but medial to the trochlear nerve is still present. On one side of the head there is a thin, densely chondrified infrapolar process which is, however, only weakly attached to the trabeculae and the basal plate. The basitrabecular process as well as its attachment to the trabecula has now chondrified. The orbital cartilages resemble a pair of wing-like structures in this stage; they lie medio-

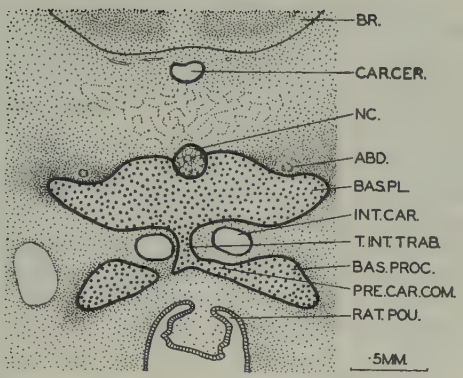


Fig. 14

Ostrich embryo 11.6 mm. Transverse section through basal plate and basitrabecular processes.

dorsal to the eyes and are broadly attached to the trabecula anteriorly. Chondrification is beginning to take place in the blasteme lateral to the abducens nerve. Up to this stage the abducens emerges from the cranial cavity through the prootic foramen. The nerve in this region lies lateral to the point of emergence of the chorda tip from the acrochordal and dorsal to the Gasserian ganglion and associated nerves. The adbucens marks the base of the pila antotica, i.e. its point of attachment to the acrochordal. In a slightly older embryo (11.6 mm.) the anterior basicranial fenestra becomes divided into right and left halves by a cartilaginous bar, the taenia intertrabecularis, separating the two internal carotid arteries. Anterior to this septum and at right angles to it there occurs a precarotid commissure separating Rathke's pouch, lying antero-dorsal to the commissure, from the arteries (Fig. 14). The precarotid commissure is not very high, and dorsal to it the carotid arteries and hypophysis lie together in the anterior basicranial fenestra. This specimen (11.6 mm.) is the only one sectioned in the present investigation to show a division of the hypophysial fenestra. In some other embryos, both younger and older, an incipient taenia intertrabecularis occurs as a small anterior outgrowth of the basal plate between the internal carotids and the point where they enter the cranial cavity.

In the 12.3 mm. stage the posterior part of the interorbital septum has increased in height owing to the chondrification of the blasteme above it (Fig. 16A). Anteriorly the ventral parts of the orbital cartilages are pressed against each other in the sagittal plane and extend upwards above the trabecula communis, to which they are attached by their ventral edges. Dorsally they diverge at an acute angle in this region to form a planum supraseptale so typical of tropibasic reptilian skulls. In this stage, therefore, the interorbital septum consists posteriorly of an unpaired dorsal derivative of the trabecula communis and anteriorly of the paired anterior roots of the orbital cartilages (Fig. 15A). The anlagen of the parietotectal cartilages of the nasal capsules have just appeared, and fuse with the anterior roots of the orbital cartilages, but in such a way that it is impossible to identify sphenethmoidal commissures. (See section on nasal capsules.) Those portions of the orbital cartilages lying behind the eyes have increased considerably in area, and project laterally beyond the otic capsules. The canal for the trochlear nerve is immediately dorsal to the pila antotica and runs dorsally

through the posterior root of the orbital cartilage to merge on its ventral surface behind and above the eye (Fig. 16B). The blasteme lateral to the pila antotica is becoming chondrified, with the result that the abducens nerve has become deeply embedded in the root of the pila antotica (Fig. 6C). Dorso-laterally to the abducens the profundus now lies in a deep indentation of that part of the pila which is covered by a ventrally directed projection (Fig. 16A), the incipient pila antotica spuria, which has not yet fused with the root of the pila antotica (vera) below the nerve. These lateral additions to the pilae antoticae give them the appearance of having moved apart, especially as the acrochordal lying between their roots is undergoing regression. The infrapolar process now joins the postero-ventral edge of the trabeculo-polar cartilage to the basal plate below the internal carotid artery, thus transforming the lateral carotid incisure into a foramen (Fig. 6C).

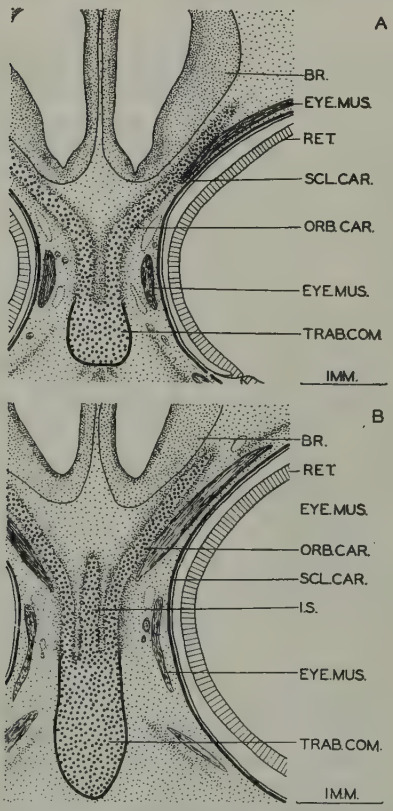


Fig. 15

Transverse sections through the interorbital septum in the ostrich.

A. 12.3 mm. ostrich embryo.

B. 15.5 mm. ostrich embryo.

C. Same embryo as B but slightly more posterior.

Owing to the growth of cartilage the floor of the pituitary fossa is almost complete in a 15.3 mm. embryo (Fig. 17B), and only a narrow opening remains ventrally. The basitrabecular process is well chondrified and no longer touches the pars quadrata as it has done in earlier stages. In this stage (15.5 mm.) and the succeeding one (17.3 mm.) the basitrabecular process appears as an equally well chondrified outgrowth of the trabecula, the

surrounding cartilage of the trabecula communis and pituitary fossa being younger and also less densely chondrified. The commissure between the suprapolar cartilage and the posterior root of the orbital cartilage has undergone regression and now consists only of dense connective tissue. The anterior ends of the suprapolar cartilages have fused with the latero-posterior surfaces of the interorbital septum (Fig. 17A). Owing to the chondrification of the mensenchyme laterally and regression mesially, the outward movement of the pila antotica continues, its relative movement being indicated by the position of the abducens. There is, however, no pila antotica spuria as yet. Regression of the orbital cartilages has begun. Dorsally the trochlear tunnel has now only a thin fibrous roof, while its ventral opening has become confluent with the sphenoid fontanelle, which is gradually encroaching on the orbital cartilages. Anteriorly, the unchondrified area in the posterior orbital cartilage described in previous stages has also become part of this general opening. The supra-orbital cartilage joining the anterior and posterior roots of the orbital cartilages has become much narrower. Posterior to the nasal capsules the orbital cartilages are intimately fused with the trabecula communis that has in the meantime grown up between their vertical ventral edges, so that the interorbital septum in this region consists of three layers (Fig. 15B). Dorsally they diverge to form the planum supraseptale. More posteriorly the orbital cartilages have only their ventral edges attached to the dorsal edge of the interorbital septum (Fig. 15C). Transverse sections of a 15.5 mm. embryo reveal that the septum posterior to this region is not a dorsal outgrowth of the trabeculae themselves but rather that these latter retain their identity as areas of denser chondrification within the general chondrification of the trabecula communis (Fig. 18). The chondrification *in situ* of the mesenchyme dorsal to the trabecula communis has resulted in the formation of the interorbital septum.

In the 17.3 mm. stage the orbital cartilages are more closely attached to the septum, although the angle at which they are attached is not as acute as in later stages. The planum supraseptale is also relatively broader, but its distal edges are already undergoing degeneration. Anteriorly, the parietotectal cartilage is extending upwards and fusing with the distal edges of the orbital cartilages and the dorsal edge of the septum to form a paired olfactory tunnel. In this stage, however, the tunnel is still short. Above the eyes, in the region of the optic nerves, the orbital cartilages have become discontinuous; they are now only connected by fibrous connective tissue. The pila antotica spuria is all but complete, being separated from the root of the pila antotica below the profundus nerve by a small space. The acrochordal has degenerated so much that the abducens now lies mesially in the root of the pila antotica in a very shallow tunnel. The pituitary fossa still opens antero-ventrally to allow passage for Rathke's pouch.

With the upgrowth of the parietotectals (in the 19 mm. stage) over the dorsal edges of the orbital cartilages and the resultant formation of the olfactory tunnels, comes a simultaneous regression of the orbital cartilages that have temporarily formed the ventro-lateral walls and floor to the tunnels. These anterior roots of the orbital cartilages have become relatively thinner and lower, their dorsal edges being only as high as the septum which lies between them. Posteriorly the tunnels still lack the parietotectal roof, and the orbital cartilages form a pair of shallow channels dorsally on either side of the septum

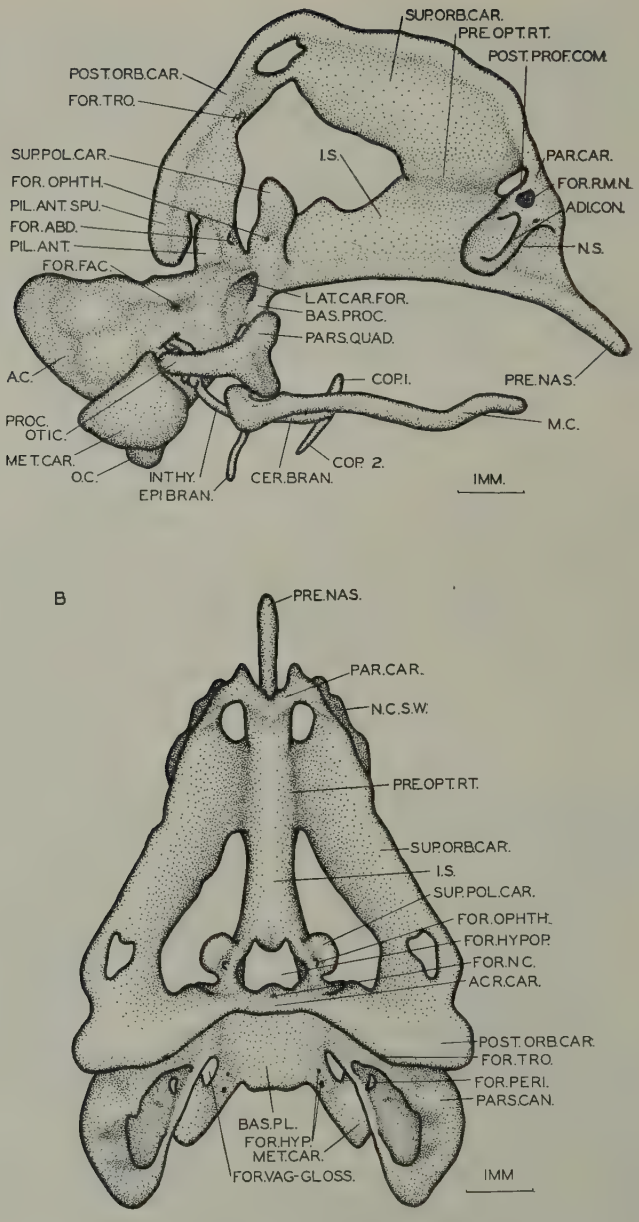


Fig. 16

Ostrich embryo 12.4 mm.

A. Lateral reconstruction of chondrocranium.

B. Dorsal reconstruction of chondrocranium.

for the olfactory nerves. Posteriorly the anterior roots of the orbital cartilages form a flat horizontal planum supraseptale ventral to the olfactory lobes (Fig. 19). The downgrowth from the pila antotica lateral to the profundus (i.e. the pila antotica spuria) has fused with the pila ventral to the nerve. All that remains of the posterior orbital cartilage is a vertically situated flange presenting a concave surface to the posterior surface of the eyeball (Fig. 8A). It has lost all contact with the anterior root of the orbital cartilage.

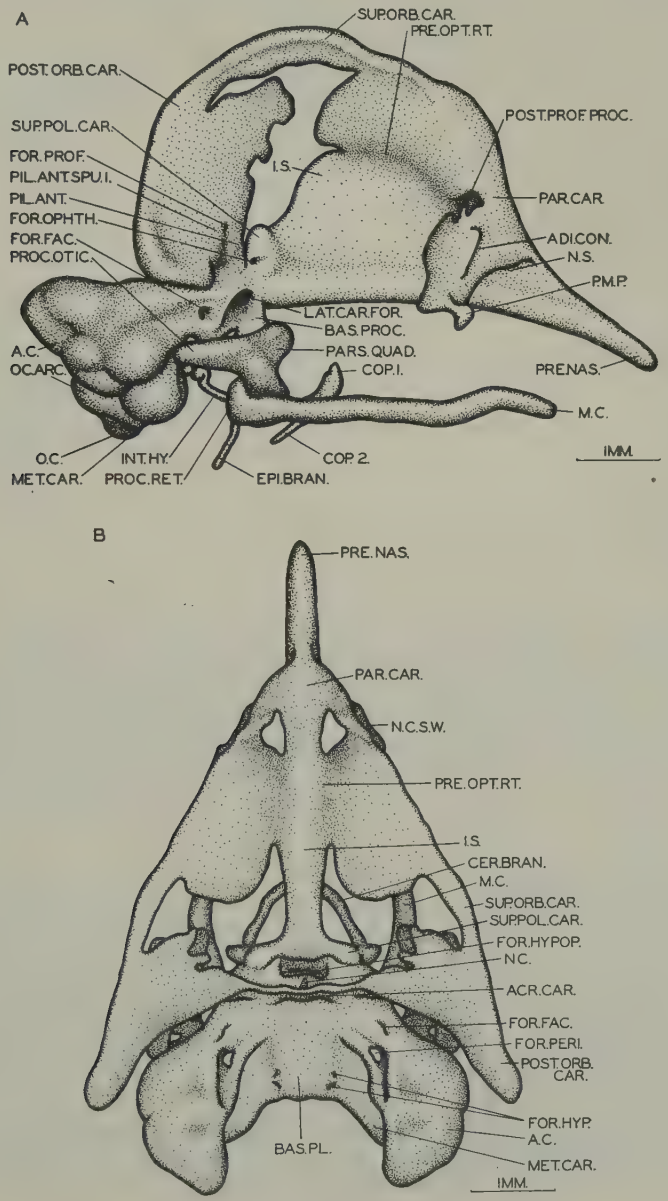


FIG. 17: Ostrich embryo 15.3 mm.

A. Lateral reconstruction of chondrocranium.
B. Dorsal reconstruction of chondrocranium.

No further development takes place in the orbital cartilage except for the consolidation of the pila antotica spuria, which becomes thicker and more solidly attached below the profundus in a 21 mm. embryo (Fig. 7A). In this stage the postero-ventral edge of the orbital cartilage has become fused with the otic capsule to complete the foramen prooticum spurium by means of the prootic process (Fig. 8B). The chondrocranium as a whole has now become homocontinuous and all trace of the separate origin of its parts is lost.

In the 21-day-old embryo a short horizontal roof overlying the anterior region of the pituitary fossa has developed from the posterior edge of the interorbital septum (Fig. 19). The optic chiasma lies immediately above this roof.

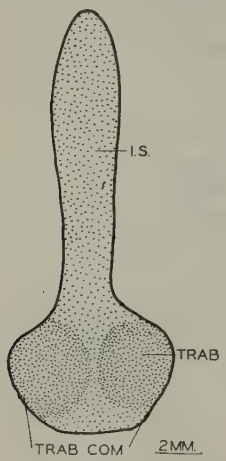


Fig. 18

Ostrich embryo 15.5 mm. Transverse section through the interorbital septum.

Ossification first occurs at the age of 34 days. In such an embryo there is slight ossification of the most anterior part of the interorbital septum as well as the parietotectal fused with the dorsal edge of the septum. The vertical flange of the orbital cartilage behind the eyes also shows signs of ossification. The only change between this and the previous stage is that the prootic process of the otic capsule is taller and is undergoing ossification.

(ii) *Discussion.*

Particularly interesting is Sonies's (1907) observation that in the duck embryo the polar cartilage becomes attached to the basal plate at the point where the cochlear part of the otic capsule (his "cartilago basiotica") fuses with the acrochordal. This condition is very similar to that in mammals, in which the alicochlear commissure fuses with the cochlear capsule instead of with the basal plate. According to de Beer (1937) this is due to the enlargement of the cochlear part of the otic capsule which replaces the basal plate in this region, but is in no way formed from it. The enlargement of the cochlear capsule in the duck has led to a similar displacement of the basal plate with similar consequences, and this phenomenon lends further support to de Beer's contention. Unfortunately Sonies's careful description of this region does not agree with that of de Beer and Barrington (1934).

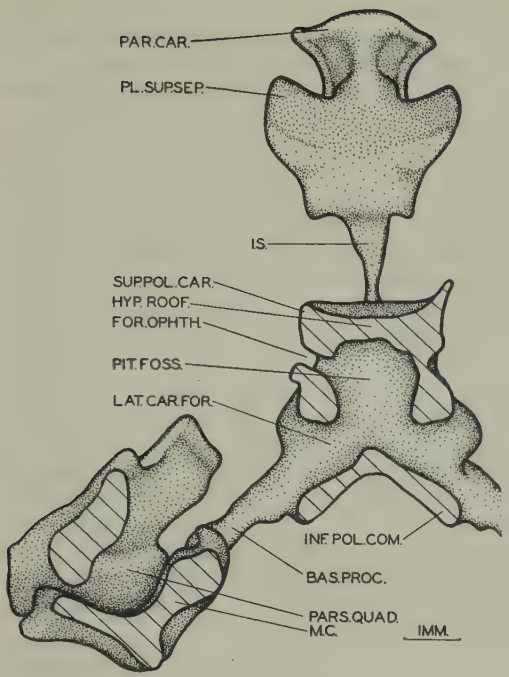


Fig. 19

Ostrich embryo 21 days. Reconstruction of interorbital septum and pituitary fossa.

In his description of the early development of the chondrocranium of the duck Sonies (1907, p. 421), referring to the pila antotica, says: "an der medialen Seite dieser Platte verursacht der N. oculomotorius einen rostralwärts offenen Einschnitt in dem dorsalen oder rostralen Rande des Acrochordale". This is probably the same notch mentioned by de Beer and Barrington (1934), which they, however, identify as occurring in connexion with the abducens nerve. Later they correctly describe the oculomotor nerve as passing dorsomesially to the pila antotica, as it does in the ostrich, while the pila antotica is pierced at its base in this stage by the abducens nerve (see figs. 46 and 47, de Beer and Barrington 1934). It is important to note that Sonies describes the oculomotor nerve in the fowl as lying between the very early anlagen of the acrochordal and the pila antotica (his lamina antotica). With the fusion of these two entities the oculomotor nerve comes to lie in a canal indicating in those forms, in which the acrochordal and pila antotica develop in continuity, the boundary between these two entities and not — as Crompton (1953) erroneously maintained — between the posterior orbital cartilage and the pila antotica. Jager (1926) confirms Sonies's findings for the fowl and describes the acrochordal in the duck as arising from the transverse commissure between the premandibular somites, while the early anlagen of the pilae antoticae appear as proliferations of their dorso-rostral parts. The pilae antoticae and the acrochordal are thus clearly separate structures and Lang's (1952, p. 128) statement that "Der Acrochordalknorpel . . . . bildet medial das Dorsum sellae und lateral beiderseits eine Pila antotica", is consequently misleading. Moreover, Jager expressly states, about the pilae

antoticae and the acrochordal, that there is no continuity between these two cartilages in the early ontogeny. There is a canal accommodating the oculomotor nerve in both the penguin and the ostrich, but it disappears with the regression of the acrochordal cartilage. Parker (1892) found a similar canal in the kiwi.

The basitrabecular process arises autochthonously in both the ostrich and the penguin (Crompton, 1953) and then chondrifies in the ostrich in continuity with the trabeculo-polar bar. In the penguin it does not chondrify completely, as the process is absent in the adult. During the early ontogeny of the ostrich the basitrabecular process touches the pars quadrata; later these two structures move apart, leaving a gap in which the os pterygoideum develops. Apparently a basitrabecular process is present in the pigeon for Filatoff (1906) describes a precartilaginous process of the pars quadrata p.q., the columella, which is fused with the trabecula. Later this process breaks away from the pars quadrata and aids in the formation of the lateral carotid foramen. It is clear that he did not consider the above process as part of the trabecula but as the anlage of the processus ascendens of the pars quadrata p.q. From his description, however, little doubt can be entertained that it is a true basitrabecular process. Lutz (1942) describes a well-developed basitrabecular process in the emu lying closely applied to but not fused with the processus basalis of the pars quadrata p.q. According to de Beer and Barrington (1934) all that remains of the basal process in the duck is a band of precartilaginous tissue stretching between the polar cartilage and the pars quadrata p.q. on each side.

As Crompton (1953) has fully discussed Kesteven's (1942) contention that the avian basitrabecular process is not homologous with that of the reptiles, and as I agree with his view it is unnecessary to repeat his arguments. However, a few additional facts brought to light in the present investigation which support Crompton's arguments must be mentioned. Kesteven believed that Brock (1937) had mistaken the ramus palatinus VII for a sympathetic fibre; but this is not so! In the ostrich, as in the penguin, the ramus palatinus VII passes from the earliest stages ventral to the basitrabecular anlage. The position of the process in relation to the nerve is thus not anomalous in these birds. Later in the ontogeny a sympathetic fibre may be seen accompanying the ramus palatinus VII, but running dorsal to the basitrabecular process. Although usual, the course of the ramus palatinus VII ventral to the basitrabecular process is not universal for the *Reptilia,* for Verslijs (1898) describes this nerve as passing dorsal to the process in *Chamaeleo vulgaris.* Kesteven's argument is confusing as he first concedes (p. 219) that "the process in the Lacertilia is correctly designated 'basitrabecular' ", while in his summary he asserts (p. 235) that "the polar cartilage is certainly not a part of the trabecula" and that "the basipterygoid process of the avian chondrocranium is a 'basipolar process' ". Strangely enough he concludes with the statement that the basipolar process is probably homologous with that of reptiles!

Although Brock (1937) maintained that there is no infrapolar process in the ostrich, this is not true, for while it is not as large or as conspicuous as in other birds, it is nevertheless present. The exaggerated precocity in the development of the infrapolar process of the ostrich reported by de Beer (1937) could not be confirmed in the present work. The confusion which has

existed in the homology of the basitrabecular and infrapolar process of birds was exhaustively discussed by Brock (1937) and Crompton (1953).

Conditions in the emu embryo described by Kesteven (1942) are exactly similar to those for the ostrich of 17 mm. where the pila antotica spuria has grown round the profundus nerve V but has not yet fused below it with the root of the pila antotica.

There seems to be no reason to doubt de Beer's (1937) homology of the avian suprapolar cartilage with the supratrabecular bar (or taenia interclinoidalis) of other forms. Its position in relation to the ophthalmic artery and oculomotor nerve is the same. Moreover, although it is free from the pila antotica in most avian chondrocrania and also in the adult ostrich, it is attached by means of a precartilaginous process to the lateral edge of the acrochordal in the early embryo. The acrochordal, although completely ventral to the pila antotica in lower amniotes, has its lateral edge continuous with the mesial edge of the pila in birds. In these amniotes extreme cranial flexure occurs, so that a shift from the base of the pila antotica to the lateral edge of the acrochordal involves very little movement. Anteriorly the suprapolar cartilage is attached to the trabecula of its side, therefore its relation to the cartilages of the side-wall of the neurocranium is also similar to that of the supratrabecular bar. Although the polar cartilage in some cases arises as an independent nodule this circumstance hardly affects its homology. No atrophy of the cartilage above the ophthalmic artery, such as occurs in the penguin (Crompton, 1953) and fowl (Sonies, 1907), was observed in the ostrich.

The precarotid commissure separating the internal carotid arteries from Rathke's pouch in the penguin is permanent. Crompton (1953) claims to have observed it in the emu but neither Lutz (1942) nor Kesteven (1942) mentions it. This commissure was observed in only one ostrich embryo (Fig. 14). Similarly the taenia intertrabecularis, which divides the hypophysial fenestra of the sparrow *(Passer domesticus,* de Beer 1937) into right and left halves, is represented in the ostrich embryo mentioned above by a transitory sagittal bar of cartilage separating the two internal carotid arteries ventrally.

Although Brock (1937) does not mention it in her account of the development of the ostrich chondrocranium, de Beer (1937) records the formation of the interorbital septum of the ostrich from discrete preoptic roots of the orbital cartilages. These are apposed and diverge dorsally to form the planum supraseptale. This mode of development resembles the ontogenetic plan for vertebrates in general formulated by Gaupp as far back as 1898. Crompton (1953, p. 88) on the other hand maintains that in the penguin "the interorbital septum and the preoptic roots are two distinct structures" that fuse later to form the definitive interorbital septum. The present investigation confirms Crompton's observations (cf. 15.5 mm. stage). although at a certain stage in the ontogeny of the ostrich (12.3 mm.) the interorbital septum actually consists anteriorly only of the ventral vertical parts of the orbital cartilages. It is possible that this independently chondrified interorbital septum, lying between the anterior roots of the orbital cartilages and distinguishable from the trabeculae, is what Suschkin (1899) identified as the "intertrabecula".

The interorbital septum of the South African night-jar embryo of approximately the same stage of development as a 21 mm. ostrich embryo is particularly heavily built. Anteriorly it is almost as broad as it is high; posteriorly it assumes more normal proportions. From the position of the olfactory nerves and plana antorbitalia it may be deduced that the latter have been forced forwards and downwards by the large eyes and come to lie almost horizontally against the septum. But it follows that that part of the septum above them is really part of the nasal septum and not part of the interorbital septum (Fig. 29C). It is this forward rotation of the planum antorbitale which is responsible for the passage of the olfactory nerve through the orbit, and results in a condition very similar to that in the salmon (de Beer, 1927). The olfactory nerves lie in a pair of shallow grooves in the sides of the dorsal extension of the septum roofed by the parietotectal cartilage. Vertically, above the posterior border of the planum antorbitale, the olfactory nerves pass through the foramen olfactorium evehens bordered ventrally by the anterior roots of the orbital cartilages, latero-dorsally by the connexion between the parietotectals and the orbital cartilages, the sphenethmoid commissures, and dorsally by the parietotectal cartilages themselves. As a result of the forward shifting of the planum antorbitale the cavum orbitonasale has become enormously elongated, and the foramen olfactorium advehens now lies stretched out between the postero-dorsal edge of the parietotectal (now lying within the orbit) and the medio-dorsal edge of the planum antorbitale. Medially it is bordered by the septum, and laterally by the commissure between the planum antorbitale and the parietotectal. The orbital cartilages are attached ventrally to the septum, but dorsally their edges diverge to form a broad planum supraseptale on which the olfactory lobes lie in the same way as in the ostrich. Posteriorly the septum is homocontinuous with the orbital cartilages. The anterior and posterior orbital cartilages have the avian pattern and are discontinuous. The posterior orbital cartilages are smaller than usual and are attached to the otic capsules by means of orbito-capsular processes (Fig. 9C). There is a well-developed pila antotica as well as pila antotica spuria (Fig. 9A). Both the posterior root of the orbital cartilage and the prefacial commissure have encroached on the foramen prooticum spurium, with the result that both the maxillary and mandibular rami of the trigeminal nerve have separate foramina, and the Gasserian ganglion lies in a fossa in the mesial surface of the pila antotica spuria. These conditions cannot be directly compared with those in mammals, in which the same result has been achieved in a different way, by the inclusion of the cavum epiptericum within the skull, the side wall being formed from the ala temporalis.

A large lateral carotid foramen is present. The suprapolar cartilage is only attached anterior to the ophtalmic artery. Posteriorly, however, it makes an extended suture with an anterior projection of the pila antotica, and the foramen thus formed encloses not only the ophtalmic artery but also the oculomotor nerve. From its relations to the artery and nerve this commissure would appear to be homologus with the temporary precartilaginous attachment of the suprapolar cartilage in the early development of the ostrich. Sonies (1907) has described a very similar cartilaginous connexion between the suprapolar cartilage and the pila antotica in the fowl and duck, and Suschkin (1899) in the kestrel. It is, however, something quite different

from the commissure between the suprapolar cartilage and the acrochordal present in the 8.4 mm. ostrich embryo; here it lies medial to the ventral opening of the oculomotor canal (Fig. 13B), which has been homologized with the supratrabecular bar. Only the most anterior part of this structure in the night-jar can possibly be compared with it. The attachment posterior to the ophthalmic artery in the ostrich seems to have disappeared in the night-jar and the floor to the pituitary fossa is not yet completed. Anterior to this fossa there is a pair of latero-ventrally directed processes articulating with the pterygoids. It was not possible to follow the course of the ramus palatinus accurately, but what appears to be the palatine nerve runs dorsal to the process, so that it is doubtful whether this represents a true basitrabecular process. The course of the abducens nerve is puzzling; anteriorly it penetrates a laterally directed ridge, to which the m. rectus externus is attached on the base of the pila antotica in a dorso-ventral direction, to lie in a shallow channel. At the end of this channel it enters the cranial cavity through a foramen in the base of the pila antotica.

(d) *THE VISCERAL ARCHES*

(i) *Description.*

It should be noted that the terms "otostapes" and "hyostapes" originally coined by Hoffman (1889) will be used entirely in a topographical and not a morphogenetic sense.

The mesenchymatous anlagen of the visceral arches first appear in a 5.4 mm. embryo. The mandibular arch is represented by a mesenchymatous crescent immediatly ventral to the basitrabecular process. The hyoid blastema clearly lying between the spiracular and second visceral (i.e. first branchial) pouch is similar in appearance. Between the second and third visceral pouches and caudad of the glossopharyngeal nerve there is a dense mesenchymatous nodule representing the first branchial arch (Fig. 20). Ventrally, in the floor of the pharynx consisting in this stage of very dense mesenchyme there is a slight cartilage matrix deposition between the cells in the region in which the copulae develop in later stages. There is no connexion between this anlage of the copulae and the visceral arches in this stage (Fig. 12A).

Fig. 20
Ostrich embryo 5.4 mm. Parasagittal section through the visceral pouches.

There is little change in the mandibular arch of the 6.8 mm. embryo, except that it is now precartilaginous. The hyoid arch is longer and appears as a dense mesenchymatous, sinuous structure with thin matrix occurring between its cells. Proximally it reaches the cochlear portion of the otic capsule, while at its most lateral point it curves sharply back so that its ventral extremity lies posterior to the mandibular arch. This blasteme of the hyoid arch shows signs of fragmentation into separate entities. The blasteme of the first branchial arch is a similarly continuous mass which now reaches the anlage of the copulae ventrally.

The mandibular arch becomes divisible into a pars quadrata and Meckel's cartilage in an 8.4 mm. embryo. The two structures chondrify separately within the mesenchymatous mandibular anlage and are connected by a wedge of dense unchondrified mesenchyme (Fig. 13A). Dorsally the pars quadrata touches the basitrabecular process directly, without the intervention of a definite basal process. Meckel's cartilage has a short processus retro-articularis; anteriorly it does not yet meet its partner. The hyoid arch has differentiated sufficiently for an "otostapes" and a "hyostapes" to be distinguished. The "otostapes" abuts against the cochlear part of the otic capsule but is not fused with it. This proximal portion is precartilaginous; distally, however, it still consists of dense mesenchyme. Ventrally, approximately halfway along its length at the point where it becomes mesenchymatous, the "otostapes" is continuous with the "hyostapes", consisting distally also of dense mesenchyme. At the point of fusion the "hyostapes" has a ventral precartilaginous interhyal process. The distal tips of both the "hyostapes" and the "otostapes" lie above each other, the former protruding laterally slightly further than the latter as the processus extracolumellaris (Fig. 2A). With advancing chondrification the mesenchymatous line of separation between the two copulae has become more distinct. They are also separated from the bases of the branchial arches by means of dense mesenchyme.

The chondrification of the otic process begins in the mesenchyme posterior to, but of course actually forming part of, the pars quadrata in a 10.7 mm. embryo. This precartilaginous process already bears against the metotic cartilage but does not reach the otic capsule. Except for the otic process the pars quadrata is now well chondrified; so is Meckel's cartilage. As chondrification proceeds the "otostapes" and the "hyostapes" become more easily distinguishable as separate entities joined by dense mesenchyme. Distally, however, each is still mesenchymatous. In this stage the second copula has developed a slender ventral process.

In the 12.4 mm. stage the pars quadrata and the basitrabecular process are no longer contiguous; the gap between them will eventually be bridged by the os pterygoideum. The tips of Meckel's cartilages still fail to meet each other anteriorly and do not reach as far forward as the prenasal process of the chondrocranium (Fig. 16A). Except for further chondrification there is no change in the hyoid arch in this stage. The strand of mesenchyme separating the two copulae is disappearing, but in contrast the branchial arch blastema is now divided into a ceratobranchial and an epibranchial.

Quite extensive changes have occurred between the previous stage and the 15.3 mm. embryo (Fig. 17A). Meckel's cartilages meet anteriorly but are not extensively fused. The anlage of the os pterygoideum is interposed

between the basal articulation of the pars quadrata and the basitrabecular process, and the otic process is now fully chondrified. The columella auris is still clearly divisible into an "otostapes" and a "hyostapes". The distal tip of the "otostapes" has become elongated to form a processus supracolumellaris medialis, curving over the posterior aspect of the columella and lying behind the processus extracolumellaris and ventral to the pars quadrata. With the rapid development of the rest of the chondrocranium the processus interhyalis has become relatively smaller. Its ventral extremity is situated close to the medial edge of the processus retroarticularis of Meckel's cartilage (Fig. 7A). The copulae are completely fused; the ventral process of the posterior copula is longer than in the previous stage. The paired paraglossal anlagen have made their appearance anterior to the copulae.

Very little further development takes place in the visceral arch skeleton. In an embryo of 21 days the anterior tips of Meckel's cartilages have fused, while the columella auris is entirely chondrified and consists of a stout rod with a broad footplate fitting into the fenestra ovalis (Fig. 8A). Distally the processus extracolumellaris is inserted into the tympanic membrane. About two-thirds of the way along its length the processus supracolumellaris medialis arises, curving round the processus extracolumellaris to lie postero-ventral to it. Its tip is likewise inserted into the tympanic membrane. A densely fibrous ligament that lies entirely within the tympanic membrane connects the distal tips of these two processes. The processus interhyalis is attached to the columella immediately ventral to the root of the processus supracolumellaris medialis. The former process runs from its point of attachment on the columella auris to another situated ventrally between the processus retroarticularis and the cranial base. From the most posterior part of the supracolumellar arcade a thin ligament runs to the latero-ventral surface of the processus oticus immediately behind the columella. This ligament is probably the same as that described by Suschkin (1899) as "Plattner's ligament". The fused copulae are now much broader, while the paraglossals are well chondrified and lie with their posterior tips, ventro-lateral to the anterior edge of the copulae. The ceratobranchial and epibranchial cartilages of the first branchial arch have become greatly elongated.

(ii) *Discussion.*

The columella auris of the ostrich is typically avian in topography. The lateral head vein, stapedial artery, and hyomandibular nerve all pass dorsal to it and mesial to the processus supracolumellaris medialis, and the internal carotid artery passes ventrally. Although embryos of all stages and both sagittal and transverse sections were examined, no trace of a chorda tympani could be found branching either from the ramus hyomandibularis or independently from the geniculate ganglion, as it does in the chick (Goodrich, 1915). The nerve which Brock (1937) identifies as the chorda tympani, but which she admits she was unable to follow, is a sympathetic fibre, which rejoins the ramus hyomandibularis just before the latter joins the geniculate ganglion. In addition it should be noted that the processus supracolumellaris medialis (Crompton, 1953), Brock's "dorsal process", is joined to the processus extracolumellaris by a tendon embedded in the tympanic membrane. Brock's description is quite different (see Brock, 1937,

p. 233 and fig. 10). The tendon probably represents the processus supracolumellaris lateralis that has failed to chondrify. In the night-jar (see later) and the penguin (Crompton, 1953) this process is similarly embedded in the tympanic membrane.

Although Crompton (1953) gives an excellent account of the homologies of the dorsal processes of the avian columella auris he says very little about the other processes. In his description of the development of the columella auris of the duck he mentions a precartilaginous interhyal connecting the stylohyal with the hyostapes in the 4½-day-old embryo. This is apparently the same structure as Sonies's (1907, Fig. A p. 469) processus infracolumellaris that serves the same purpose. (The interhyal, according to de Beer (1937) is also known as the infrastapedial). Of interest at this point is Crompton's claim that at a certain stage in the development of the penguin the processus infracolumellaris is found together with an independent blastematous connexion between the stylohyal and the columella auris. Thus this "infracolumellar process" of the penguin cannot be considered homologous with that of the duck. No autochthonously developed ventral process was found in the ostrich; from the time it first appears in the ontogeny it is continuous with the hyostapedial anlage. For this reason it is considered to be an interhyal and not a stylohyal. Brock (1937) designated this ventral process of the columella auris of the ostrich an interhyal (her infrastapedial), but it would seem from her description that she considered the process to include a stylohyal. Its length certainly seems to indicate this. (Holmgren (1943) equates the stylohyal with the interhyal in his studies on the head of fishes). It is not certain what de Beer (1937) means by the "intercalary" which he describes in the supracolumellar arcade of the ostrich, and Brock (1937) makes no mention of such a structure. The various nomenclatures favoured by workers on avian ontogeny is bound to be confusing, and it is quite impossible at this juncture to attempt to create order from so much diversity. There is moreover the vexed question of an exact homologization of columellar process of birds with those of living diapsid reptiles.

In support of Crompton's contention (1953) that the paraglossal cartilages (Kallius, 1905), or the ceratohyals (de Beer and Barrington, 1934) are morphogenetically "new" structures, it may be mentioned that the very fact that they develop so much later than the hyoid arch in the ontogeny

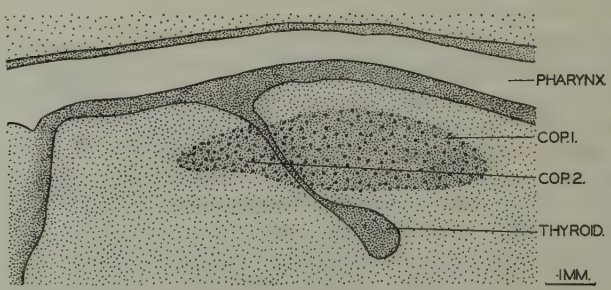


Fig. 21

Ostrich embryo 5.4 mm. Parasagittal section through anlage of copulae and thyroid gland.

of both the ostrich and the penguin seems to indicate that they are not derivatives of this arch. The paraglossals of the ostrich arise and remain separate entities during ontogeny; they do not fuse with each other as they do in both the penguin and the duck.

Very early in the ontogeny of the penguin Crompton (1953) finds a connexion between the hyoid blastema and that of the anterior copula, thus proving that the anterior copula is a basihyal. No such connexion was found in the ostrich. However, the earliest anlage of the copula in the ostrich (Fig. 21) is pierced by the stalk of the developing thyroid gland in much the same way as this stalk pierces the basihyal of *Scyllium* (de Beer, 1937). This is strong evidence in favour of Crompton's view.

The visceral arches of the night-jar embryo of roughly the same stage of development as a 21 mm. ostrich embryo are typically avian. Anteriorly Meckel's cartilages are in close contact but are not fused with each other. The pars quadrata lacks a basal process while the otic process is supported by both the crista parotica of the otic capsule and the metotic cartilage (Fig. 9B). It also contacts the ventro-lateral edge of the orbital cartilage in the same way as in *Pyromelana* (Engelbrecht, in press), the duck and fowl (Sonies, 1907). The columella auris has a supracolumellar arcade presumably consisting, as it does in the duck, of a processus supracolumellaris lateralis and p.s. medialis, the former lying within the tympanic membrane (Fig. 9C). There is, also a processus extracolumellaris inserting into the tympanum. The position of the stylohyal and the tortuous path followed by the cartilaginous rod connecting it with the columella auris seem to indicate the presence of a connecting interhyal, though no such structure could be demonstrated. The paraglossal cartilages of the night-jar embryo reach the anterior tip of the fused copulae. The ceratobranchial and epibranchial are apparently fused, although it is possible that their actual point of separation was overlooked as only transverse sections were available.

(e) THE NASAL CAPSULES

(i) *Description.*

Since detailed descriptions of the extensive gyrations exhibited by the "turbinals" tend only to confuse, contour section-drawings of the nasal capsules are given instead, descriptions being curtailed to the few points of interest. As the nasal capsules of the South African night-jar and of the rhea, which are also dealt with in this section, have never been fully described before, a short description of each is included.

In the ostrich the medial walls of the conchae nasales, that is the more medial portions of the paranasal cartilages, are the first parts of the nasal capsules to make their appearance as a pair of dense layers of mesenchyme lying laterally to the nasal cavities and still completely isolated from the rest of the chondrocranium. This occurs in the 10.7 mm. stage.

In the next stage (11.6) the paranasals, now slightly chondrified, are attached by means of dense connective tissue and the post-profundal commissures, first described by Crompton (1953) in the penguin, to the dorsal roots of the anterior orbital cartilages. In front of these commissures the ethmoidal nerves divide into their two components.

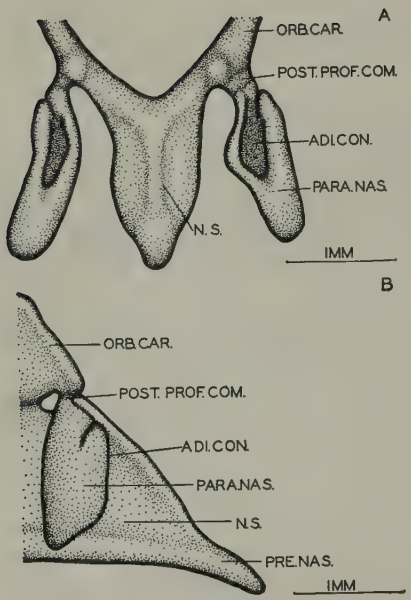


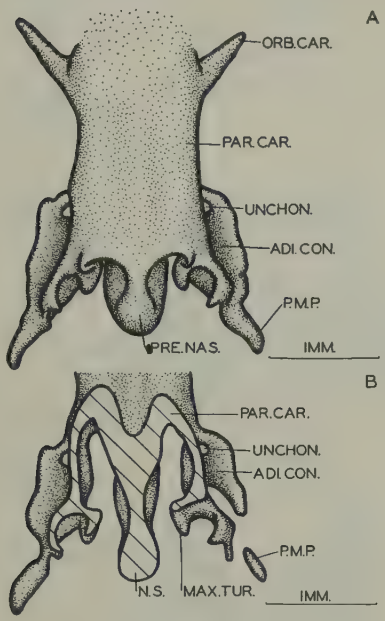
Fig. 22

Ostrich embryo 12.3 mm.
A. Anterior reconstruction of nasal capsules.
B. Lateral reconstruction of nasal capsules.

The paranasal cartilages are completely formed in the 12.3 mm. stage, the cava conchalia having received their outer or more lateral walls (Fig. 22A and B). Chondrification is still very slight, and these elements of the capsules still consist mainly of dense mesenchyme with only the slightest deposition of intercellular matrix. The position of the roof of the capsules and the maxilloturbinals by this time is faintly indicated by a mesenchymatous condensation. All these components of the nasal capsules are formed in syndesmotic and later synchondrotic continuity with what is already present. At no stage in the development could independent elements be demonstrated. It was thus impossible to identify separate parietotectal or antorbital elements or to determine the exact role they play in the formation of the capsules. It is assumed that the outer wall of the cavum conchale consists of the outer wall of the paranasal cartilage intimately fused with the planum antorbitale, as is the case in the duck (de Beer and Barrington, 1934). Similarly the position of the sphenethmoid commissures is obscure. The olfactory nerve passes out of the cranial cavity through a fissure bordered posteriorly by the front edge and preoptic root of the orbital cartilage, medially by the nasal septum, and laterally by the parietotectal and post-profundal commissure. Anteriorly the fissure is open, to be closed later by the backwardly growing parietotectals.

All the basic elements of the nasal capsules are chondrified by the time the 15.5 mm. stage is reached (Fig. 17A). The plana antorbitalia are well developed, although they still have areas of incomplete chondrification near their upper borders (Fig. 23C). The paranasal and parietotectal cartilages have grown forwards, thus deepening the conchae, while the roof of the capsules is well formed anteriorly (Fig. 23A). The parietotectals are now growing backwards linking the free dorsal edges of the orbital cartilages.

They will eventually form the olfactory tunnels. In this way each foramen olfactorium evehens is covered over, and the sphenethmoidal commissures become indistinguishable from the parietotectals. On the postero-ventral edge of the planum antorbitale there is an extremely well-developed processus maxillaris posterior pointing in a ventro-lateral direction (Fig. 23A). The maxilloturbinals are represented in this stage by small curved processes on the inside of the parietotectals, their concavities facing sideways (Fig. 23B). The prenasal process extends anteriorly far in front of the nasal capsules.



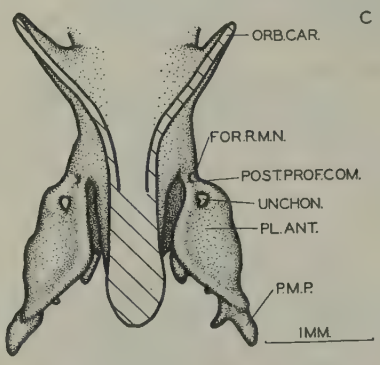


Fig. 23

Ostrich embryo 15.5 mm.

A. Anterior reconstruction of nasal capsules.
B. Reconstruction of portion of the nasal capsules.
C. Posterior reconstruction of nasal capsules.

Owing to the growth of both the orbital and septal cartilages the olfactory nerves now lie in separate olfactory channels leading from the cranial cavity to the nasal capsules. These channels are formed by the anterior roots of the orbital cartilages, which are attached ventrally to the interorbital septum that has grown up between them thus dividing the space into right and left halves. In this stage the post-profundal commissure has degenerated on one side of the capsule. However, the foramen through which the ramus medialis enters the capsule is now completed anteriorly by the upgrowing parietotectal that has fused with the orbital cartilage and the medial paranasal wall. Just in front of this there are two small nerve foramina in the side wall antero-dorsal to the aditus conchae.

In the 17.3 mm. stage both post-profundal commissures are present and well developed. The capsules have undergone little change since the last stage, except anteriorly where they are further chondrified. The atrio-turbinals are now evident as dense mesenchymatous structures lying lateral to the more anterior portions of the maxilloturbinals. The latter have become divided ventrally into secondary lamellae each of which curls dorsad. The

maxilloturbinals are still more or less mesenchymatous anteriorly. Anterior to the conchae and lateral to the roots of the maxilloturbinals there is a pair of long narrow antero-posteriorly running fissures in the side-walls.

Except for a few small details the configuration of the fully formed nasal capsules is established in the 19 mm. stage. There is still no chondrification in the atrioturbinals, although they are larger than in the previous stage. The parietotectals have grown backwards forming a roof to the olfactory tunnels in which the olfactory nerves lie. Posteriorly these tunnels are roofed over by connective tissue alone. Meanwhile the floor to the tunnels is disappearing in front with the recession of the ventral edges of the anterior roots of the orbital cartilages where they reach the interorbital septum.

In the nasal capsule of a 21-day-old ostrich embryo, the prenasal process is extremely well developed and extends as a rod from the ventral edge of the septum. Neither septum nor parietotectal is fenestrated, but

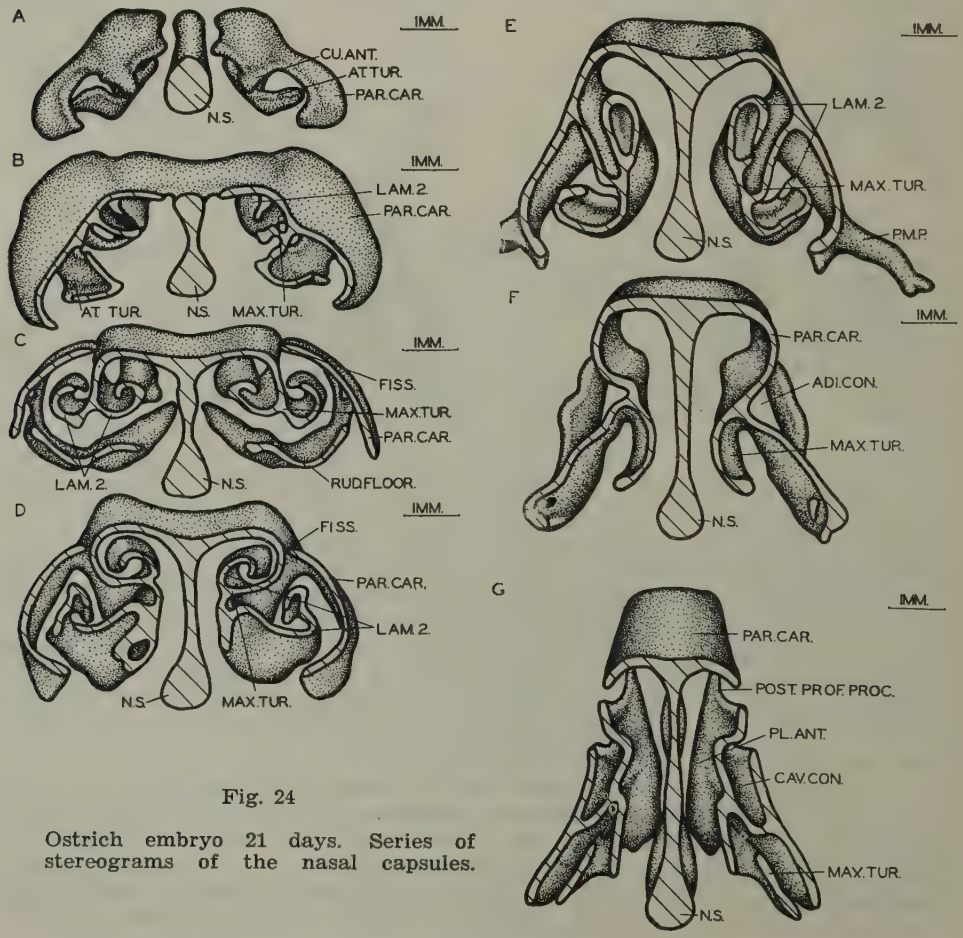


Fig. 24

Ostrich embryo 21 days. Series of stereograms of the nasal capsules.

lateral to the bases of the maxilloturbinals, at the point where the atrioturbinals pass over into the side-walls, there are longitudial areas of incomplete chondrification indicating the lines of attachment of the atrioturbinals to the bases of the maxilloturbinals (Fig. 24C and D). The side-walls of the capsules at this point are actually derivatives of the atrioturbinals. Such an extra side-wall to the capsule is absent in many forms and this results in the exposure of the maxilloturbinals laterally (e.g. duck, *Pyromelana* and penguin). The conchae lie dorso-lateral to the maxilloturbinals and do not form a floor to the capsules (Fig. 24F and G). Anteriorly there are small ventrally directed processes of the parietotectals which probably represent the cupolae anteriores (Fig. 24A).

The parietotectal roof over the olfactory nerves now extends as far as the planum supraseptale (Fig. 19) but the floor to the tunnels has completely atrophied, with the result that the nerves now appear to lie within the orbital chamber. Both maxilloturbinals and atrioturbinals are extremely well developed and exhibit many convolutions; anteriorly their roots are confluent (Fig. 24B). As it passes over into the side-wall the atrioturbinal has a ventral, medially directed, triangular process which almost reaches the septum (Fig. 24C). This process is the only floor to the capsule. Owing to the mode of attachment of the maxilloturbinal to the planum antorbitale there is a peculiar "double posterior wall" to each capsule (Fig. 24G). This turbinal has become most elaborate, its ventral edge dividing anteriorly to form secondary lamellae, each of which curves upwards through about half a whorl. The concha nasalis is comparatively small, is pressed into the general surface of the capsule, and contains a diverticulum of the nasal cavities. The post-profundal commissures that in earlier stages connected the parietotectals to the orbital cartilages have now lost their dorsal connexions and only remain as dorsally directed processes behind the nerves (Fig. 29A). Of the two foramina antero-dorsal to the aditus conchae in the 15.5 mm. stage only one is now present. The processus maxillaris posterior of the antero-lateral edge of the planum antorbitale, though relatively smaller than in previous stages, is well chondrified (Fig. 24E).

Very few significant differences were observed between a 21 and a 34-day-old embryo. The maxilloturbinals have undergone further elaboration, their surfaces becoming more irregular and the edges of the secondary lamellae dividing for a short distance to form tertiary lamellae (Fig. 25A). Ventrally, on either side of the septum nasi near the back of the capsules, a pair of small flattened cartilaginous bars have appeared; judging from their position posterior to the vomers they are apparently paraseptal derivatives (Fig. 25B). A certain amount of ossification has taken place in the septum and in the roof of the capsules posteriorly.

(ii) *Discussion.*

Many of the differences between the nasal capsules of the ostrich and those of other birds can be explained by assuming that the capsules have been laterally compressed. In contrast with most birds it must be noted that in the ostrich the nasal capsules lie between the eyes (which are enormous, especially in early ontogeny) rather than wholly anterior to them. Owing to this compression the recessus extraconchalis, which is typically present in the lizard but small in the duck, is quite absent in the ostrich, for the parietotectal is fused with the outer wall of the concha and thus obliterates

the recessus. The concha bulges out in the duck (de Beer and Barrington, 1934) and has its opening directed more or less laterally. In the ostrich compression has displaced it into the capsule, and it now almost faces forwards. It is diffcult to see what Brock (1937, p. 232) means when she says that it "divides the capsule into anterior and posterior chambers". It is noteworthy that should the nasal capsule of *Pyromelana* (Engelbrecht, in press) be compressed laterally (the eyes of *Pyromelana* lie posterior to its nasal capsules) one would get an almost point for point reduplication of the posterior regions of the nasal capsules of the ostrich. Thus the planum antorbitale, well-developed in *Pyromelana,* would then become reduced to a size comparable with that of the ostrich. Ventrally a double posterior wall to the capsule would be formed by the maxilloturbinal pressing on the planum antorbitale.

In her attempt to identify the turbinals of the ostrich from Parker's (1866) rather involved description, Brock (1937), has confused his "middle turbinal" with a secondary elaboration of the maxilloturbinal. Parker's description of the nasal turbinals of the falcon (1876, p. 134) is easier to understand: he simply states that "the middle turbinal is merely represented by an irregular ridge on the fore face of the antorbital plate". Further, in his work on the ostrich (1866) he also finds a fold on the base of the planum antorbitale that he identifies as a middle turbinal. Unfortunately his figure illustrating this is difficult to orientate and it was not possible to determine exactly what he had in mind. That the structure referred to by Brock is not the same as Parker's middle turbinal may be gathered from his description (1866, p. 127) of the maxilloturbinal (his inferior turbinal): "where the aliseptal (parietotectal) cartilage begins to turn downwards it sends off an outgrowth (maxilloturbinal) which is directly vertical behind, and afterwards turns inwards: at this anterior part it splits into two lamellae, which curl upwards, each being about a semicircle. But posteriorly these secondary lamellae are divided again; and of these tertiary folds, the nearest but one to the septum forms more than a complete coil." (Terms in parenthesis inserted by author; cf. this description with Fig. 25A).

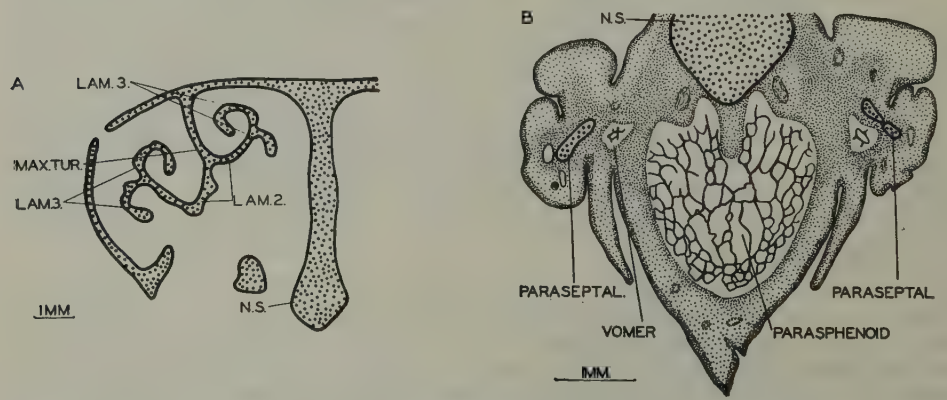


FIG. 25: Ostrich embryo 34 days.

A. Transverse section through nasal capsules.
B. Transverse section through paraseptal cartilages.

Because of the numerous connecting sympathetic fibres it was not possible to identify the r. medialis and r. lateralis nasi of the ethmoid nerve. Nevertheless, it would appear that posterior to the concha the nerve divides into its two components. In the 15.5 mm. stage the r. medialis nasi enters the capsule through a foramen whose posterior wall is formed by the postprofundal commissure that in previous stages was the first connecting link between the orbital and parietotectal cartilages. The r. lateralis nasi, after leaving the r. medialis, is continued anteriorly without entering the capsule above the aditus conchae. Anterior to the point where the r. medialis enters the capsule there occur two more foramina, through which run commissures between the r. medialis and r. lateralis. It is just possible that one of these commissures represents the r. lateralis, in which case the foramen through which it leaves the capsule would be the foramen epiphaniale. In the 21-day stage only one of these foramina remains. Unfortunately earlier in the ontogeny, when the sympathetic fibres are not present, the nasal capsules are still incomplete so that it is difficult to express an opinion on the true state of affairs. De Beer (1937) in his description of the ontogeny of the ostrich chondrocranium identifies the opening as the foramen epiphaniale. He states (p. 287): "the ramus lateralis of the profundus nerve which is now caught up in the cartilage forming the antero-lateral border of the foramen olfactorium advehens, emerges from the capsule through an epiphanial foramen".

De Beer and Barrington's (1934) explanation of the curious intraorbital course of the olfactory nerve in birds is fully substantiated by observations made in the earlier stages of the ontogeny of the ostrich as well as *Pyromelana:* its position is purely secondary owing to the disappearance of the anterior orbital cartilages in the older stages as well as to the upgrowth of the parietotectals on the upper edge of the interorbital septum.

In his description of the nasal capsules of the ostrich de Beer (1937, p. 286) says: "The hind wall of the capsule, or lamina orbitonasalis, forms a cupola posterior and presents a convex surface to the orbit". This is a contradiction of Brock's findings (1937), on whose work de Beer has based his description. Brock (1937, p. 288) notes that the "planum artorbitale forms a flat posterior wall"; she then reiterates (p. 282) that "the planum antorbitale has the flattened appearance due to the enlargement and pressure of the eyeball". A wax plate model made by me has concave surfaces towards the orbits. This condition is doubtless due to the large eyes of the ostrich; they bulge out on either side of the nasal capsules. The posterior portions of the latter lie between, rather than anterior to, the eyes. This condition is exaggerated in the younger stages where the eyes are relatively even larger.

Although Brock (1937, figs. 4 and 7) illustrates ventro-laterally directed processes of the plana antorbitalia in her work on the ostrich she does not give any explanation of them. Crompton (1953) was the first to suggest that these might be homologous with the processûs maxillares posteriores of reptiles. I find that these processes and the processûs maxillares posteriores of *Lacerta* have a similar topography. This conclusion is based on the relations of the dermal bones of this region and also of the nasolacrymal duct (Fig. 30A and B). It must be admitted that the course of the nasolacrimal duct is rather veriable in tetrapods but its relations to

the nasal capsule within the amniotes (excepting mammals) is nevertheless constant and may be considered as lending support to the above contention.

The relationship of the cartilago uncinata of the penguin to the blasteme of the pars pterygoidea led Crompton (1953) to believe that the former was also homologous with the processus maxillaris posterior of reptiles. No trace of this blasteme occurs in the ostrich. But in the earlier embryonic stages the process is better chondrified than the wall of the nasal capsule to which it is attached, which supports the theory that it has a different origin.

In the adult ostrich the processus maxillaris posterior becomes ossified. Distally it is firmly attached to the ventral edge of the prefrontal (lacrymal) and proximally it is free in the dried skull. As it is delicate it is possible that its connexion with the ectethmoid has broken down during maceration. Pycraft (1900, p. 179) describes a bone, the "ossiculum palatinum, which in the adult fuses with the lacrymal on the one hand, and the antorbital plate on the other". Later in the same work (p. 204) he again refers to it, this time as the ossiculum lachrymo-palatinum and qualifies his previous statement by saying that it articulates "mesially, in very old specimens, with the antorbital plate". There can be no doubt that the ossicle is the same as that referred to above, i.e. an ossification of the processus maxillaris posterior. However, it was first described and termed the os uncinatum by Brandt in 1840. His term thus has precedence over Pycraft's. No reference to this ossicle could be found in Parker's work on the ostrich (1866), although he does describe it in several other birds. A similar process is present in the adult rhea, but it is not ossified. Crompton (1953) maintained upon comparative-morphological grounds that the os uncinatum is homologous with an os substitiens arising in the processus pterygoideus of fishes (so-called os autopalatinum). Since the intermediate étappes between the bone in birds and fishes have not been demonstrated, it is a possibility to be reckoned with that the os uncinatum of birds is a phylogenetically "new" structure. Consequently it has been decided to retain the specific avian nomenclature for the bone.

There is a close similarity between the nasal capsules of the adult rhea and those of the ostrich, but the capsules of the rhea are simpler. Unfortunately, owing to the condition of the material, it was not possible to reconstruct the most anterior part of the capsules. The atrioturbinals resemble those of the ostrich and pass over in exactly the same manner into the side-wall of the capsule and cover the maxilloturbinals laterally, though not as completely as in the ostrich (Fig. 26A and B). Their lines of attachment are indicated by a series of small fenestrae at the bases of the maxilloturbinals (Fig. 26C and D). Anteriorly the maxilloturbinals are separate from the atrioturbinals and are less complex in structure than those of the ostrich. The paired paraseptal cartilages are broad and lie latero-ventrally to the nasal septum. Posteriorly they narrow down to thin bars of cartilage (Fig. 26C). Pycraft (1900) refers to these paraseptals as Jacobson's cartilages and describes them as lying immediately dorsal to the vomers. He states that W. K. Parker (1866) was the first to describe them in the rhea. The small paired cartilaginous nodules, which lie in a similar position in the ostrich, are probably also vestiges of the paraseptals. These two birds together with the kiwi (Parker, 1891) and the tinamou (de Villiers, 1946) are the only birds for which the remnants of paraseptals have been described.

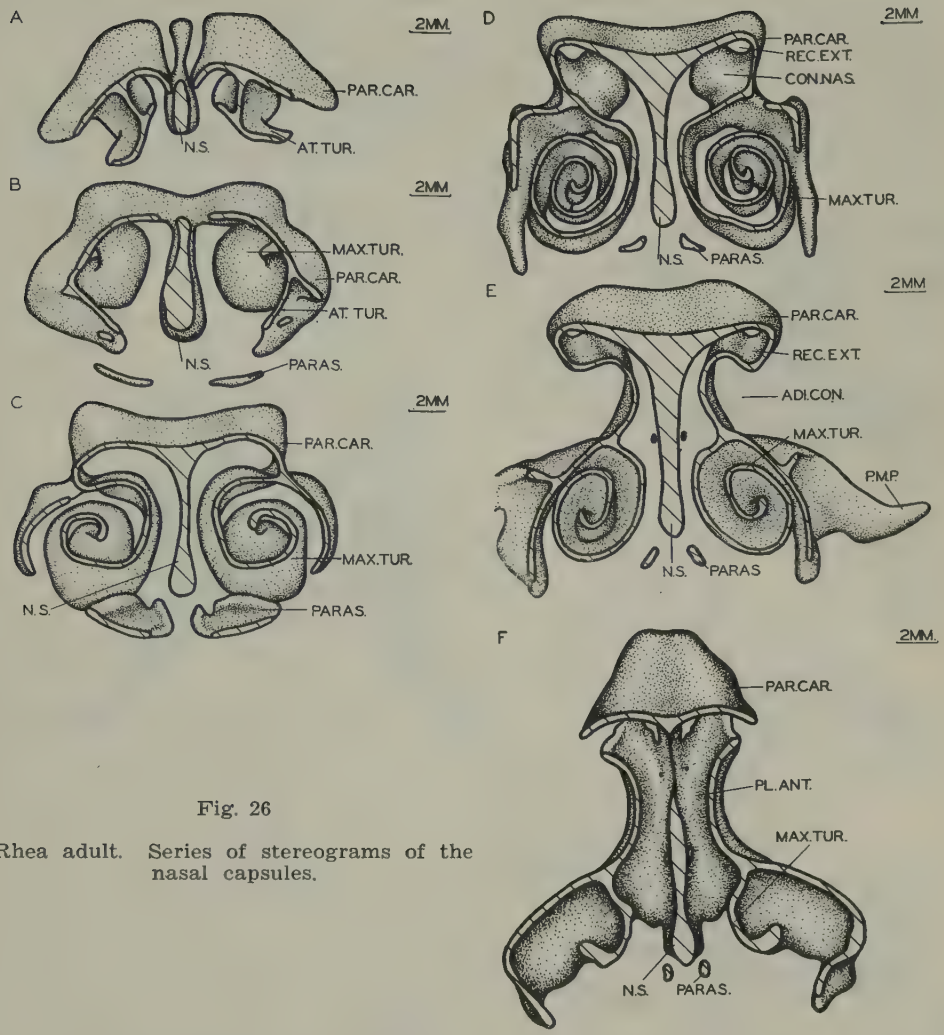


Fig. 26

Rhea adult. Series of stereograms of the nasal capsules.

The concha of the rhea has a very peculiar arrangement, but this is merely due to the more ventral position of the anterior edge of the planum antorbitale. The concha (Fig. 26E and F), instead of lying anterior to the planum antorbitale, now lies above it, as in the night-jar. In the rhea the conchal pocket in the side-wall is very well developed. The basin-like depression faces latero-posteriorly and lacks posterior protection by means of the antorbital cartilage as in other forms. The aditus conchae is obstructed by a large glandula lateralis nasi. Owing to the more postero-dorsal position of the eyes there is little lateral compression of the capsules, consequently the recessûs extraconchales are situated dorso-lateral to the conchae. Antero-ventral to the conchae the plana antorbitalia bear large laterally projecting

processûs maxillares posteriores (Fig. 26E). The posterior walls of the capsules have the same double appearance as in the ostrich though they are not as pronounced. Here, as in the ostrich, this is due to the peculiar fusion of the maxilloturbinals with the antorbitalia (Fig. 29B). The ethmoid nerve divides before entering the capsule and there is nothing even vaguely reminiscent of a foramen epiphaniale. The r. medialis nasi also runs ventrolaterally to the post-profundal process before entering the capsule. A small

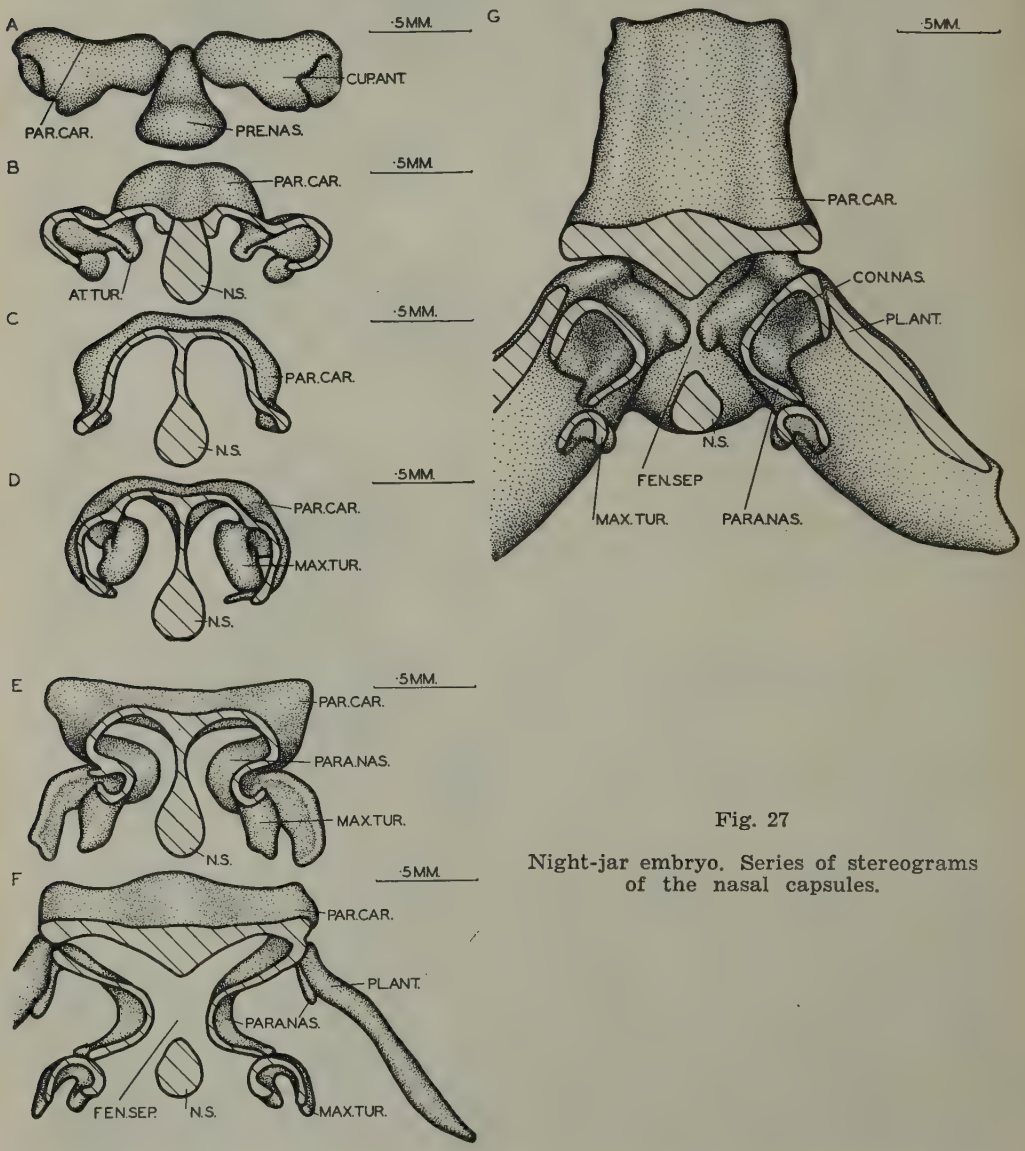


Fig. 27

Night-jar embryo. Series of stereograms of the nasal capsules.

pair of cartilaginous rods of doubtful significance must be mentioned; they lie in the posterior third of the capsules in close proximity to the septum (Fig. 26E and F), but separated from it by connective tissue. They probably represent portions of the plana antorbitalia such as are found in the night-jar where they form small "antorbital turbinals". Anteriorly the septum nasi is unfenestrated but posteriorly it has two openings, separated by a thin vertical bony lamella. The septum is extensively ossified posteriorly, as are also the parietotectals towards the back of the capsule.

In order to elucidate the conditions in the nasal capsules of the ostrich and the rhea still further a study was made of these structures in a South African night-jar embryo (corresponding approximately in development to a 21 mm. ostrich embryo), as well as in a nestling of the same bird. This proved most fruitful as the various elements of the nasal capsules were still separate although the embryo was quite far advanced. Both a maxilloturbinal and an atrioturbinal, as well as a concha nasalis, are present, and the planum antorbitale is extraordinarily well developed.

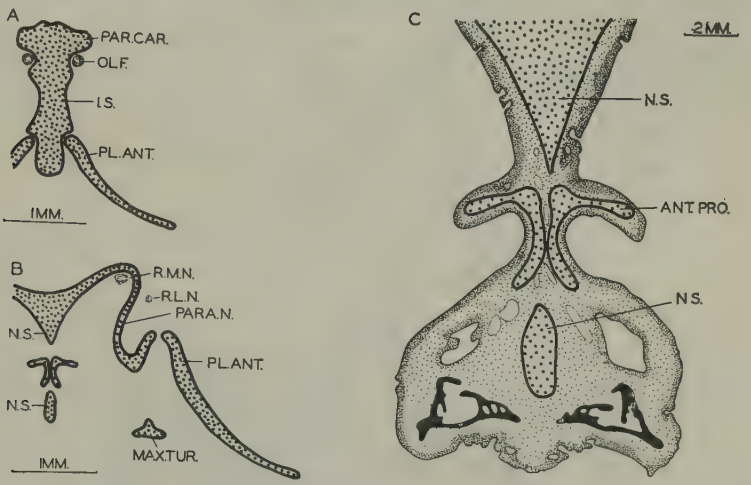


Fig. 28
Transverse sections through nasal capsules of the night-jar.
A. Embryo of night-jar.
B. Nestling of night-jar.
C. Nestling of night-jar.

In the embryo the atrioturbinal arises as an invagination of the roof of the capsule (Fig. 27B). This is shallow anteriorly, but becomes progressively deeper until in its posterior part its edges are confluent. On the ventral surface of the anterior parietotectal it appears as a solid ridge. The maxilloturbinal on the other hand is formed by an infolding of the ventral edge of the parietotectal (this point is more clearly illustrated in *Pyromelana*, duck and fowl) curved in such a way that the concavity faces sideways and downwards (Fig. 27D and E). Laterally the maxilloturbinal is not protected by the atrioturbinal which especially in the rhea and the ostrich extends posteriorly to meet the parietotectal side-wall and in this way

encloses the maxilloturbinal laterally. Posteriorly the entire concha nasalis is formed by a distinct element, the paranasal (Fig. 27F and G). In the night-jar this cartilage lies compressed (cf. de Beer and Barrington 1934, fig. 17C) between the parietotectal, represented by the posteriorly elongated maxilloturbinal, and the planum antorbitale. It must be noted, however, that whereas in the duck the planum antorbitale lies posterior to the concha, in the night-jar owing to the size of the eyes and their peculiar position (they lie dorso-lateral to the plana antorbitalia), the planum antorbitale has rotated forwards and now lies more or less dorsal to the paranasal. The result is that the latter is dorso-ventrally depressed (Fig. 29C), rather than antero-posteriorly, as in the duck. The relative shifting of the planum antorbitale is most clearly illustrated by its relation to the interorbital septum (Fig. 28A). The planum, instead of appearing as a vertical plate on either side of the septum, now lies horizontally at an angle of about 45° to the septum with its medio-dorsal edge touching the ventral surface of the septum. There is a large fenestra in the posterior part of the nasal septum.

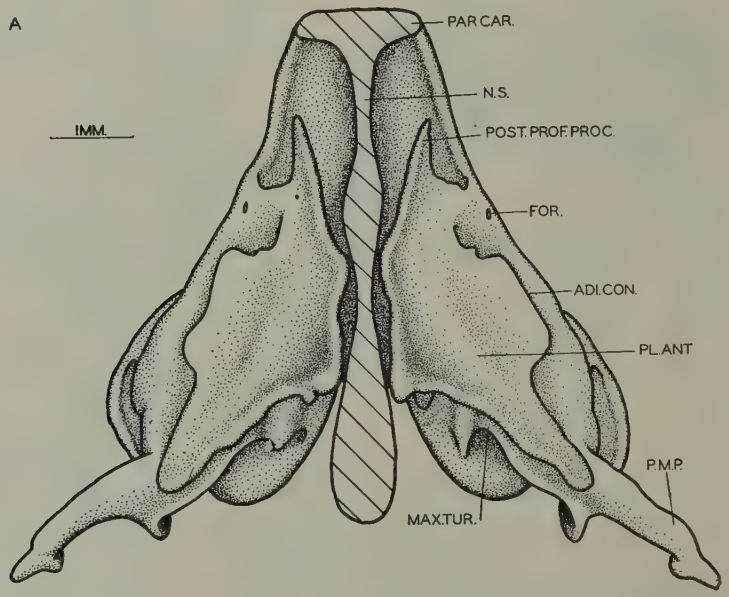


Fig. 29

Posterior reconstruction of the nasal capsules of:

A. Ostrich embryo 21 days.

In the nestling of the night-jar the long prenasal process, as well as the more anterior part of the septum, both of which were present in the embryo, have disappeared. The parietotectal has come to form a laterally directed funnel surrounding the external naris. Farther back in the region of the maxilloturbinal and at about the same level there appear a few isolated cartilaginous nodules lying lateral to the capsule. Similar nodules are present

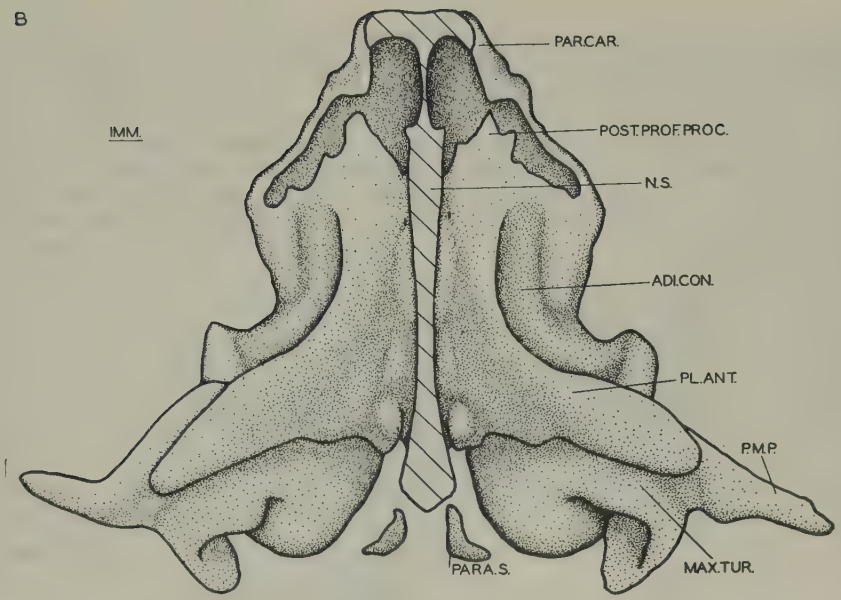


Fig. 29

Posterior reconstruction of the nasal capsules of:
B. Rhea adult.

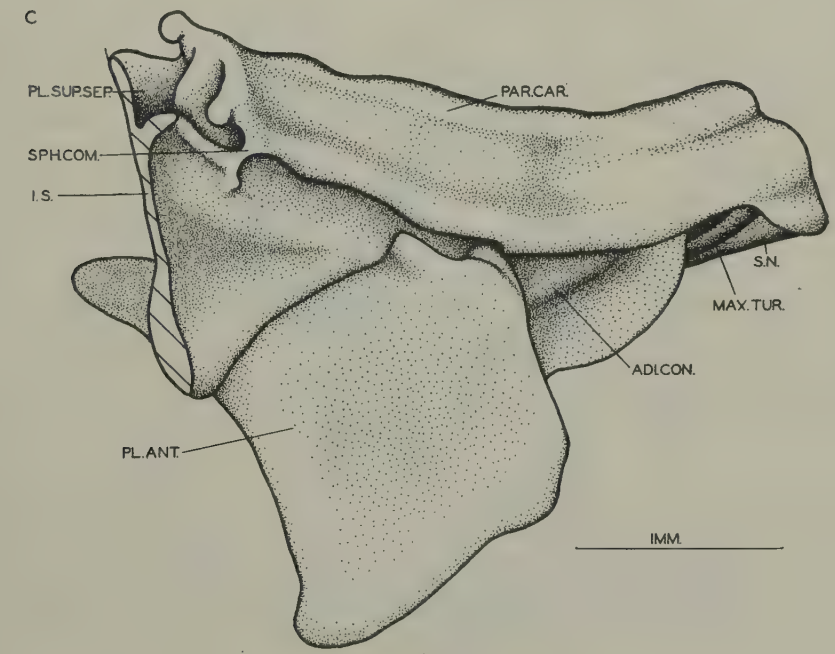


Fig. 29

Posterior reconstruction of the nasal capsules of:
C. Night-jar embryo (Postero-dorsal view).

in *Pyromelana* (Engelbrecht, in press) but their exact significance is unknown. The paranasal cartilages are no longer separate elements, as the lines of attachment to the parietotectals and plana antorbitalia have disappeared. The planum antorbitale also lies lower with respect to the paranasal whose ventral edge is now attached to the antero-dorsal edge of the former. Instead of being compressed below the planum, the concha now lies almost above it with the aditus facing laterally. This condition (Fig. 28B) is strongly reminiscent of that in the rhea, where the concha also lies above rather than in front of the planum antorbitale. The plana antorbitalia give

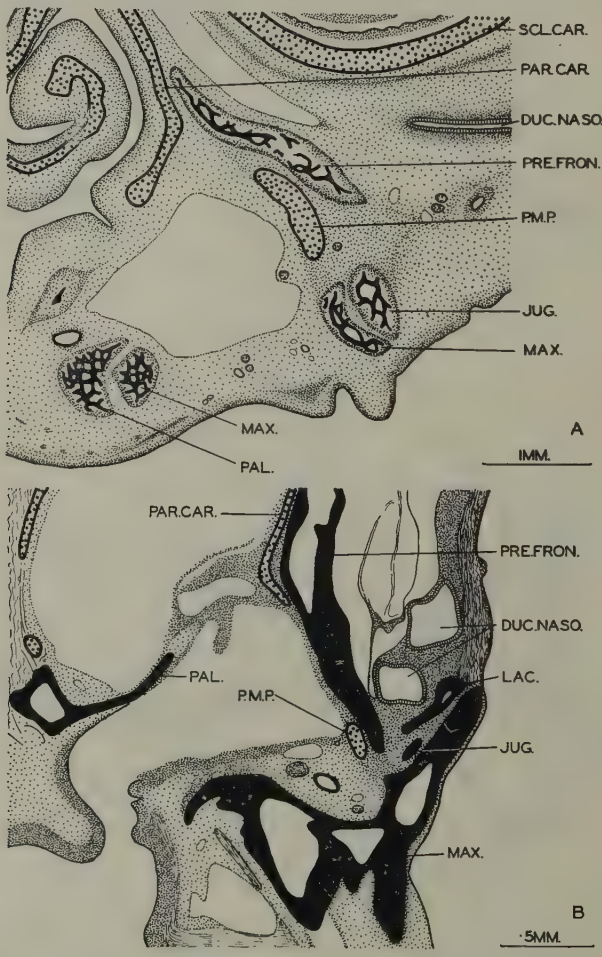


Fig. 30

Transverse sections showing topography of processus maxillaris posterior in:

A. Ostrich embryo 21 days.
B. *Lacerta* adult.

off a pair of anteriorly directed processes appearing in section as inverted "L's" lying against each other in the fenestra septi, which is filled with connective tissue (Fig. 28C). The processes are covered with nasal epithelium and their function is probably similar to that of the other turbinals. Of interest is a small median, unpaired cartilaginous bar situated anteriorly beneath the septum of the nestling. A similar nodule known as the cartilago papillae palatinae is present in certain mammals.

The nasal capsules of the ostrich, night-jar and *Pyromelana* illustrate three possible solutions to the problem of the accommodation of the enormously enlarged eyes which became essential to the proavian form with the transition from a terrestrial to an arboreal-aerial mode of life. Simultaneously the olfactory sense diminished in importance with the result that the orbits encroached upon the space available to the nasal capsules. In the ostrich the eyes have bulged out on either side of the capsules thus compressing them laterally. In the night-jar on the other hand the eyes lie dorsal to the capsules, with the result that these have been displaced ventrally. Comparison of the nasal capsules of *Pyromelana* (Engelbrecht, in press) with those of the ostrich reveal that the former are relatively more compressed in their posterior regions than is the case in the ostrich, presumably by the eyes which lie directly behind them.

V. CONCLUSION

As de Beer (1937) has shown, the intensive study of the avian chondrocranium has served only to emphasize the close relationship between birds and reptiles, and between birds and crocodiles in particular. The points of affinity have been tabulated and thoroughly analysed by him. Hofer (1952) considers the large size of the brain and eyes, coupled with the loss of the olfactory sense and tropytrabism, the chief characteristics which distinguish the avian from the mammalian skull. Gaupp (1906) has suggested that the large brain and eyes have been responsible for the extreme cranial flexure as well as the ventral position of the otic capsules, and in particular the pars cochlearis. There can be little doubt that the size of the brain and the eyes are responsible for several lesser phenomena, such as the compression of the posterior regions of the nasal capsule, the breaking down of the supraorbital cartilages, and the subsequent passage of the olfactory nerve through the orbit. The contact of the posterior orbital cartilage with the otic capsule by means of either a short prootic process or an orbitocapsular process or of both, and the contact of this cartilage with the pars quadrata in some birds, probably also fall within this category. Moreover, it is probable that cranial flexure plays quite an important rôle in the formation of the infrapolar connexion of the trabecular part of the chondrocranium with the basal plate.

The monophyletic origin of *Aves* and the polyphyletic origin of the *"Ratitae"* originally propounded by Fürbringer (1888) in his monumental work on birds has recently received support from such workers as Stresemann (1927) and de Villiers (1946). The latter considers it inconceivable that dromaeognathic birds can be a truly monophyletic group. Lowe (1928, 1933 and 1942) on the other hand consistently maintains that the *"Struthiones"* are descended from a common prevolant ancestor. It is interesting to note that

recently Craigie (1940, p. 227), after extensive research into the cerebral cortex of birds comes to the conclusion: "its character and distribution, however, are basically uniform and present no evidence in support of the view that palaeognathine and neognathine birds may have had a separate phylogenetic origin".

It is now universally accepted that the *"Ratitae"* represent a lower phase, anatomically speaking, than the *"Carinatae"*. Further as Lowe has more than adequately proved, and as has been confirmed by the present investigation, the *"Ratitae"* are not degenerate or retrogressive. However, while Lowe believes that the *Struthiones* (ostrich and its allies) are truly primitive, both de Beer (1937 and 1940) and de Villiers (1946) have suggested that the *Dromaeognathae* are neotenic. De Villiers has, however, pointed out that it is notoriously difficult to distinguish beween a neotenic and a truly primitive condition. De Villiers (1934, p. 410) has defined neoteny as follows: "if occasionally a member of a group in the adult form retains organs in a condition characteristic of the ontogenetic condition of other members of the group; or if in the adult anatomy of the said member, mutual relations of organs are such as prevail in the ontogeny of other members of the group, the said member displays neotenic features": He also pointed out that neoteny may be restricted to a certain organ or organs, in which case partial neoteny results. Such is the case mentioned by de Beer (1940) of the plumage of the ostrich and other flightless birds and penguins: it resembles the nestling down of young nidicolous birds. The same explanation must be given of the persistent sutures between the bones in many dromaeognathic birds. De Villiers (1946) has also pointed out that large size is often indicative of a neotenic condition. Many of the larger amphibians such as *Pipa, Xenopus, Cryptobranchus* and *Megalobatrachus* are considered neotenous. It is thus quite possible that the same may be true of the *Dromaeognathae* which are the largest birds. De Beer (1937, p. 446) has shown that it has almost universally been assumed that since all the "neognathous" palate types can be formally derived from the "palaeognathous" and that because neognathous birds pass through a palaeognathous-like stage in their ontogeny the palaeognathous types must of necessity be primitive. This "dogmatic application of the theory of recapitulation is now discredited".

On comparing the ostrich chondrocranium as representative of the *"Ratitae"* with that of a typical "Carinate", such as the duck or perhaps better still *Pyromelana,* it soon becomes clear that the ostrich chondrocranium is typically avian, and does not possess any features which can be labeled as more reptilian than those present in the *"Carinatae"*. It may not be out of place to mention a few characters which at first sight appear to be primitive but which also appear in some *"Carinatae"*. In the ostrich the pila antotica spuria is late in appearing in the ontogeny and is never very robust. But this spurious pila has been shown by Kesteven (1942) and Brock (1937) not to be invariably well developed in the higher birds (e.g. *Podiceps* and *Irideparra).* Crompton (1953) considered the presence of a processus supracolumellaris medialis in the ostrich to be a primitive characteristic, but as this process is present in conjunction with the processus supracolumellaris lateralis to form "Huxley's foramen" in many birds, it is very doubtful whether it can be considered as truly primitive. Although a processus maxillaris posterior of the nasal capsule has never been described in the *"Carinatae"*,

the uncinate ossicle (ossification of this process) has been found in many species, so that it seems that this reptilian feature is not restricted to the ostrich and rhea. As *Pyromelana* (Engelbrecht, in press) has a far better developed solum nasi and paraseptals than the ostrich, the presence of paraseptals in the latter cannot be held to be evidence of primitiveness Suschkin (1899) describes a well-developed floor to the vestibulum in hawks. The only truly reptilian feature in the ostrich chondrocranium is the continuous interorbital septum while the cranio-facial fissure is absent on account of the greatly restricted dromaeognathous type of kinetism. However, Parker (1891) has described what he considers to be a transitory cranio-facial foramen in the kiwi embryo (cf. de Villiers, 1946).

It is interesting to note that Lowe (1928) believes that the evolution of the struthious palate has been arrested in an "embryonic" stage, and again that the characters which constitute the general make-up of the *Struthiones* represent, practially invariably, primitive or almost "embryonic" characters indicative of an early phase in the evolution of the true bird. The above statements are particularly significant when it is remembered that Lowe has repeatedly rejected the theory of the neotenic origin of the *"Ratitae"*.

Although it is suggested that the ostrich is neotenic, it does not mean that it is entirely without specializations; on the contrary, as de Villiers (1934) has shown, neoteny is itself an important type of specialization. The high degree of development in the nasal turbinals is clearly another case in point.

Thus although the present work has produced no fresh, positive evidence in favour of the theory that the ostrich is neotenic, it has been shown that the chondrocranium possesses no characteristics that can be considered as being more truly primitive than those found in the *"Carinatae"*. It seems reasonable to conclude that if the ostrich were truly primitive, it would have borne the reptilian imprint in its chondrocranium more clearly than has been found to be the case.

VI. SUMMARY

1 The acrochordal cartilage arises after the perichordal plate is already established.
2 The fenestra basicranialis posterior is absent.
3 During early ontogeny the oculomotor nerve is enclosed in a canal occurring in the region where the pila antotica passes over into the acrochordal.
4 The hypocentra of at least two absorbed occipital vertebrae are represented.
5 The hypocentra of only the axis and atlas develop beyond the precartilaginous condition.
6 There are never more than three hypoglossal nerve roots present.
7 The metotic cartilages appear as independent anlagen, early in the ontogeny.
8 Three transitory cranial rib anlagen are present, but they do not contribute to the formation of the metotic cartilage.
9 The glossopharyngeal and vagus nerves have a common foramen.
10 The cochlear portion of the otic capsule arises in continuity with the basal plate.

11 The processus lateralis partis cochlearis fuses ventral to the facial nerve with the canalicular part of the otic capsule.
12 The prefacial commissure lies between the canalicular portion of the otic capsule on the one hand and the basal plate and cochlear portion of the otic capsule on the other.
13 The ventral part of the cochleo-canalicular fissure is absent owing to a precocious outgrowth of the cochlear portion of the otic capsule.
14 The foramen ovale appears as a result of resorption of the capsular wall.
15 The medial roof is the last part of the otic capsule to chondrify.
16 The tectum posterius and tectum synoticum are apparently fused.
17 The prootic process connects the otic capsule with the posterior orbital cartilage, thus completing a foramen prooticum spurium.
18 A crista parotica of the otic capsule supports the otic process of the pars quadrata.
19 The polar cartilages do not arise autochthonously.
20 From the earliest stages the suprapolar cartilage is attached to the trabeculo-polar bar.
21 The basitrabecular process arises as an independent mesenchymatous anlage dorsal to the palatine nerve.
22 Anteriorly the ventral parts of the orbital cartilages are fused with a dorsal outgrowth of the trabecula communis, to form the interorbital septum.
23 The more dorsal parts of the anterior orbital cartilages form the planum supraseptale.
24 In the later ontogeny the orbital cartilages become discontinuous dorsally, above the eyes.
25 The pila antotica spuria which arises as a downgrowth of the posterior orbital cartilage is weakly developed.
26 With the degeneration of the orbital cartilages the olfactory nerves come to lie topographically within the orbit.
27 The interorbital and nasal septi are not fenestrated.
28 The pars quadrata has no distinct basal process, but there is a well-developed otic process.
29 The early anlage of the hyoid arch is continuous, but later it fragments into an "otostapes" and a "hyostapes".
30 Copula 1 and copula 2 of the first two visceral arches appear in the early ontogeny as two independent anlagen that later fuse.
31 The first branchial arch is segmented into a ceratobranchial and an epibranchial.
32 The columella auris has a well-developed processus interhyalis. Both the processus extracolumellaris and p. supracolumellaris medialis insert on the tympanic membrane.
33 The chorda tympani is absent.
34 The paranasal cartilage is the first part of the nasal capsule to make its appearance. No independent planum antorbitale has been observed in this investigation.
35 Owing to the mode of development no sphenethmoid commissures arise.
36 The atrioturbinals and maxilloturbinals are exceptionally well developed.

37 The parietotectal cartilages grow backwards linking the dorsal edges of the orbital cartilages to form temporary "olfactory tunnels".
38 Processûs maxillares posteriores are present and comparatively large; they ossify as "ossa uncinata".
39 Small paraseptal derivatives are present lying on either side of the nasal septum.
40 In early ontogeny the paranasal anlagen are attached to the orbital cartilages only by the post-profundal commissures.

VII. ABREVIATIONS USED IN FIGURES

ABD.	Abducent nerve
A.C.	Auditory Capsule
ACC.	Accessory nerve
ACR. CAR.	Acrochordal cartilage
ADI. CON.	Aditus conchae
ANL. A.C.	Anlage of auditory capsule
ANL. BAS. PROC.	Anlage of the basitrabecular process
ANL. COP.	Anlage of copula
ANL. TEC. SYN.	Anlage of tectum synoticum
ANT. PRO.	Anterior process of planum antorbitale
AT. PLEURO.	Atlas pleurocentrum (Proc. odontoideus)
AT. TUR.	Atrioturbinal
BASI. DORS.	Basidorsal of atlas
BAS. PL.	Basal plate
BAS. PROC.	Basitrabecular process
BR.	Brain
1. BRAN. ARC.	First branchial arch
CAR. CER.	Carotis cerebralis
CAV.	Cavity of pars cochlearis
CAV. CON.	Cavum conchale
CAV. MET.	Cavum metoticum
CER. BRAN.	Ceratobranchial
COC. FLOOR.	Floor to pars cochlearis
COCH.	Cochlea
COL. AUR.	Columella auris
CON. NAS.	Concha nasalis
COP. 1.	First copula
COP. 2.	Second copula
CRAN. RIBS.	Cranial ribs
CR-VERT. FISS.	Cranio-vertebral fissure
CU. ANT.	Cupola anterior
D.C-C.C.	Dorsal cochleo-canalicular commissure
DIEN.	Diencephalon.
DOR. GAN. 1.	Dorsal root ganglion of 1st cervical nerve
DOR. GAN. 2.	Dorsal root ganglion of 2nd cervical nerve
DUC. NASO.	Ductus nasolacrimalis

EPI. BRAN.	Epibranchial
EYE. MUS.	Eye muscle
FAC.	Facial nerve
FEN. SEP.	Fenestra in nasal septum
FISS.	Longitudinal fissure in parietotectal
FOR.	Foramen above aditus conchae
FOR. ABD.	Foramen for the abducent nerve.
FOR. ACU.	Foramen for the acustic nerve.
FOR. END.	Foramen for ductus endolymphaticus
FOR. FAC.	Foramen for the facial nerve
FOR. HYPOP.	Foramen hypophyseos
FOR. MAG.	Foramen magnum
FOR. NC.	Foramen for the tip of the notochord
FOR. OCC.	Foramen for the oculomotor nerve
FOR. OPHTH.	Foramen for the ophthalmic artery.
FOR. OVAL.	Foramen ovale
FOR. PERI.	Foramen perilymphaticum
FOR. PROF.	Foramen for the profundus nerve
FOR. PRO. SPU.	Foramen prooticum spurium
FOR. R.M.N.	Foramen for the ramus medialis nasi
FOR. TRANS.	Foramen transversarium
FOR. TRO.	Foramen for the trochlear nerve
FOR. VAG-GLOSS.	Common foramen for the vagus and glossopharyngeal nerves
FOSS.	Fossa for the Gasserian ganglion
F.PL.	Footplate of the columella auris
G. GAS.	Ganglion Gasseri
G. GEN.	Ganglion geniculatum
G. JUG.	Ganglion jugulare
GLOSS.	Glossopharyngeal nerve
G. NOD.	Ganglion nodosum
G. PET.	Ganglion petrosum
G. SUP.	Ganglion superius
H. AT.	Hypocentrum of atlas
H. AX.	Hypocentrum of axis
H. OC.	Hypocentrum of first absorbed occipital vertebra
H. PRO. A.	Hypocentrum of proatlas
HYP.	Hypoglossal nerve
HYP. FOR.	Hypoglossal foramen
HYP. ROOF.	Hypophysial roof
HYP. ROOTS.	Hypoglossal roots
INC. MET.	Incisura metotica
INC. PRO.	Incisura prootica
INF. POL. COM.	Infrapolar commissure
INF. POL. PROC.	Infrapolar process
INT. CAR.	Internal carotid artery
INTER. FISS.	Intervertebral fissure
INT. HY.	Interhyal

INTRASCLER.	Intrasclerotomic constriction
I. S.	Interorbital septum
JUG.	Jugal
LAC.	Lacrymal
LAM. 2.	Secondary lamella
LAM. 3.	Tertiary lamellae
LAT. CAR. FOR.	Lateral carotid foramen
LAT. CAR. INC.	Lateral carotid incisure
L. H. V.	Lateral head vein
MAX.	Maxillary
MAX. TUR.	Maxilloturbinal
M. C.	Meckel's cartilage
MES. CON. 1.	Mesenchymatous connexion between suprapolar cartilage and pila antotica
MES. CON. 2.	Mesenchymatous connexion between suprapolar cartilage and acrochordal
MESEN.	Mesencephalon
MET. CAR.	Metotic cartilage
METEN.	Metencephalon
MID. EAR. CAV.	Middle ear cavity
MYELEN.	Myelencephalon
VIII. N.	Acustic nerve
NC.	Notochord
N.C.S.W.	Nasal capsule side-wall
N. HYP.	Notch for the hypoglossal nerve
N. PROF.	Notch for the profundus nerve
N. S.	Nasal septum
N. TRI.	Notch for the r. mandibularis and r. maxillaris of the trigeminal nerve
O. C.	Occipital condyle
OC. ARC.	Occipital arch
OCC.	Oculomotor nerve
OLF.	Olfactory nerve
ORB. ART.	Orbital artery
ORB. CAR.	Orbital cartilage
ORB. PROC.	Orbito-capsular process
O. VES.	Otic vesicle
PAL.	Palatine
PARA. NAS.	Paranasal cartilage
PARAS.	Paraseptal
PAR. CAR.	Parietotectal cartilage
PAR. CAN.	Pars canalicularis
PARS. COCH.	Pars cochlearis
PARS. QUAD.	Pars quadrata
PIL. ANT.	Pila antotica
PIL. ANT. SPU.	Pila antotica spuria
PIL. ANT. SPU. I.	Incipient pila antotica spuria

PIT. FOSS.	Pituitary fossa
PL. ANT.	Planum antorbitale
P. L. P. C.	Ventral portion of processus lateralis partis cochlearis
PL. SUP. SEP.	Planum supraseptale
P. M. P.	Processus maxillaris posterior
POL. CAR.	Polar cartilage
POST. ORB. CAR.	Postorbital cartilage
POST. PROF. COM.	Postprofundal commissure
POST. PROF. PROC.	Postprofundal process
PRE. CAR. COM.	Precarotid commissure
PRE. FAC.	Prefacial commissure
PRE. FRON.	Prefrontal
PRE. NAS.	Prenasal process
PRE. OPT. RT.	Preoptic root of the orbital cartilage
PRO. AT. PLEURO.	Proatlas pleurocentrum (Occipital condyle)
PRO. EXT. COL.	Processus extracolumellaris
PROC. OTIC.	Processus oticus
PROC. PRO.	Prootic process
PROC. RET.	Processus retroarticularis
PROC. SUP. COL. MED.	Processus supracolumellaris medialis
RAT. POU.	Rathke's pouch
REC. CON.	Recessus extraconchalis
RET.	Retina
R. HY.	Ramus hyomandibularis
RIDGE.	Precartilaginous ridge
R. L. N.	Ramus lateralis nasi
R. M. N.	Ramus medialis nasi
R. PAL.	Ramus palatinus
R. PROF.	Ramus profundus
RUD. FLOOR.	Rudimentary floor
SCL. CAR.	Sclerotic cartilage
SEG. 8-12	8th to 12th segment
SP. GANG.	Spinal ganglion
SP. N. 1-4	1st to 4th spinal nerve
STY.	Stylohyal cartilage
SUP. ORB. CAR.	Supraorbital cartilage
SUP. POL. CAR.	Suprapolar cartilage
TEC. SYN.	Tectum synoticum
TELEN.	Telencephalon
T. INT. TRAB.	Taenia intertrabecularis
TRAB.	Trabeculo-polar bar
TRAB. COM.	Trabecula communis
TRI.	R. maxillaris and r. mandibularis of the trigeminal nerve
UNCHON.	An unchondrified area
VAG.	Vagus nerve
VAG. & ACC.	Vagus and accessory nerves
VIS. POU. 2-4	2nd to 4th visceral pouches

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